FINAL REPORT

The Potential Effects of the Red Imported Fire Ant (*Solenopsis invicta*) on Survivorship of Monarch Butterfly (*Danaus plexippus*) Eggs and Larvae in Northeast Texas

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Abstract

This study documented the survivorship of spring generation monarch eggs and larvae to the third instar in Texas and evaluated the effects of Red Imported Fire Ants (*Solenopsis invicta*) (RIFA), host plant arthropod communities, and host plant characteristics on monarch survival. Preliminary data on the survivorship of fall generation eggs and larvae in north Texas is also provided.

Spring survivorship of unmanipulated monarch eggs varied from 10% to 14% in the three years of this study, despite considerable variation in weather conditions. These values were higher than similar studies conducted elsewhere despite the fact that RIFA density was above the average mound densities reported for the U.S.A. Monarch mortality was unrelated to distance of the host plant to nearest RIFA mound, the number of mounds adjacent to host plants, and the volume of mounds adjacent to host plants. Eggs on host plants with low numbers of RIFA had much higher survival than eggs on host plants with many RIFA or eggs on host plants with no RIFA. RIFA only ascended a host plant in large numbers when there was a high overall abundance of arthropods on the plant or a predictable food resource.

Direct effects of RIFA on monarch survival were measured by manipulating the density of RIFA on and adjacent to the host plants. Artificially drawing RIFA onto the host plants decreased the survivorship of monarch eggs and larvae. When chemical treatments were used to reduce RIFA populations, the effect on monarch survival was minimal despite the almost complete elimination of RIFA from the treated area. RIFA suppression had no effect on survival in the first year and only a slight positive effect on survival in the second year. This suggests that compensatory predation occurred in the absence of RIFA.

Asclepias.viridis host plants were occupied by a rich and dynamic arthropod community. Of 86 types of arthropods recorded, 10 were milkweed specialists and 28 were predators. Monarch depredation was opportunistic and subject to indirect effects. Increased numbers of non-predatory arthropods improved the survivorship of monarch eggs and larvae. Furthermore, there were density dependent effects; as the number of non-predatory arthropods increased, predator pressure decreased, and monarch survival increased. However, the positive effect of non-predatory arthropods on monarch survival was

most evident on host plants with high predator pressure. These findings suggest that complex community level indirect effects occurred on the host plants. These effects include the influence and types of alternate prey species and intra-guild predation.

Evaluation of terrestrial arthropods around host plants in the control and RIFA suppressed treatments did not yield strong predictive models of monarch survival. However, in 2018, when the overall abundance of arthropods was low, more groups of arthropods affected monarch survival than in the preceding year when arthropod abundances were high. This highlights the importance that species diversity has on the ability of the community to buffer predation in the event of population fluctuations.

Despite the fact that many plants suffered pathological symptoms, these symptoms had little effect on the arthropods occupying the plants or on the survival of monarch eggs and larvae. Larger plants were more likely to support more arthropods and favored higher survival of monarchs.

The cardenolide content of *A viridis* host plants was within the range reported for this species elsewhere. There was no effect of cardenolide content on arthropod abundance or on monarch survival. There was no evidence of cardenolide induction in response to monarch herbivory and no evidence that monarch females selected host plants based on cardenolide content.

This study is the first to document the survival of fall monarchs in the southern U.S.A. Survivorship of fall monarchs was lower than for spring monarchs in Texas, but comparable to survivorship recorded in the northern U.S.A. There was no evidence that fall monarchs compete with queens for milkweed resources. Fall monarch productivity in Texas may represent an important contribution to overwintering populations in Mexico.

The results of this study indicate that control of RIFA in Texas is unnecessary in most cases. Management activities that increase floral diversity and milkweed regeneration are more effective means of improving monarch productivity for both spring and fall populations.

Introduction

In 2014 the monarch butterfly (*Danaus plexippus plexippus*) was petitioned for listing under the Endangered Species Act (ESA) (Monarch ESA Petition 2014). This came in response to 90% declines in populations of monarch butterflies east of the Rocky Mountains in the previous decade (Monarch ESA Petition 2014). Listing of the monarch butterfly under the ESA has enormous economic ramifications across this species' near continental distribution because protections provided under the ESA have major influences on land use, land management, and development. Critically important to the listing of a species under the ESA, and the protections delineated by that listing, is the quality and extent of scientific information regarding that species. The purpose of this study was to document the potential role that Red Imported Fire Ants (RIFA) (*Solenopsis invicta*) have on the survivorship of monarch eggs and larvae in northeast Texas.

The eastern population of the monarch butterfly in North America has a near continental distribution that covers the area east of the Rocky Mountains to the Atlantic Ocean in Canada and south into central Mexico (Scott 1986). The species is migratory, spending the winter in mountain refuges in central Mexico and migrating north in spring. Spring migration is accomplished through successive reproductive events; a first generation that occurs in the southern tier of the U.S., a second generation that occurs across the central U.S., and a third and fourth generation that occurs in the northern tier of the U.S. and southern Canada. This breeding distribution encompasses more than 12 million km^2, though only a portion of this breeding distribution may be active at any given time (Flockhart et al. 2013). Population size is lowest during the winter due to an extended period of predation and mortality without reproductive recruitment (Malcolm et al. 1993). Because of this, first generation recruitment in spring is extremely important for establishing the size of subsequent generations. Recent isotopic analyses have shown that the most important portion of North America for the production of first generation adults is in Texas and Oklahoma (Flockhart et al. 2013).

Despite the important role that north Texas plays in reproductive recruitment of the monarch butterfly, there is little information on what factors affect spring reproduction in this region. Studies in other areas report that monarch survival rates from egg to fifth instar are extremely low, as low as 4% in Louisiana but more generally ranging from 5% to 20% across the species' breeding distribution (Prysby and Oberhauser 2004). Survivorship curves of monarchs in Wisconsin demonstrate that most mortalities occur within seven days of the eggs being laid and, in some cases, there was 50% mortality within the first 24 hours (Prysby 2004). In Minnesota, it was found that only 20% of eggs survived long enough to hatch into 1st instar larvae (De Anda and Oberhauser 2015). Mortality rates among larvae beyond the first instar tends to be lower.

One study in central Texas showed complete reproductive failure (0% survival), a result that was attributed to depredation by RIFA (Calvert 1996). None of the 61 eggs survived past the first instar. A follow-up study using exclosures to exclude fire ants and other terrestrial predators found survivorship rates of 1.6% to 27% inside the exclosures and 0 to 1.4% outside the exclosures (Calvert 2004). These results strongly suggest that RIFA have an important impact on monarch reproductive success in Texas.

RIFA are known to have negative impacts on at least some vertebrates (Kopachena et al. 2000, Allen et al. 2004) and are well known to have negative community-wide impacts on arthropod populations (Porter and Savignano 1990, Morrison 2002). However, there is also evidence that some arthropods may benefit from the presence of RIFA (King and Tschinkel 2006) and, in some cases, there is a positive relationship between RIFA density and arthropod diversity (Morrison and Porter 2003). This can occur if RIFA influence trophic cascades as found in one species of tropical ant (Dyer and Letourneau 1999) and could also occur if RIFA had negative impacts on other predators of monarch eggs and larvae.

The studies conducted in Texas (Calvert 1996, 2004) suggest that RIFA have important negative impacts on monarch reproduction. However, the 1996 study was based on a crude correlation between high RIFA mound density at the study site, a single observation of a RIFA attacking a first instar larva, and complete reproductive failure based on only 61 eggs. The follow-up study, which used exclosures,

provides stronger support for the idea that RIFA are important predators on monarch eggs and larvae (Calvert 2004). That study, based on over 700 eggs, found monarch survivorship was 26 times higher inside the exclosures than outside the exclosures and RIFA densities were 3.4 times higher outside the exclosures than they were inside the exclosures. However, the study still did not isolate RIFA as the cause of higher mortalities outside the exclosures because the effect of the exclosures on other predators was not measured. There are myriads of other arthropods that prey on monarchs, including wasps, spiders, stink bug nymphs, syrphid fly larvae, ladybird beetles, assassin bugs, lacewings, and variety of other dipterans (De Anda and Oberhauser 2015, Oberhauser et al. 2015). Lastly, ants other than RIFA, are known as important predators of monarch eggs and larvae (Prysby 2004) and the study by Calvert (2004) did not indicate whether predation rates were higher than would be expected from native ants. To understand the role RIFA play in the survivorship of monarchs in Texas, it is necessary to understand RIFA predation in the larger context of the host plant and the dynamics of the arthropod communities on and around the host plant.

The purpose of this study was to investigate the potential role that RIFA play in the survivorship of monarch egg and larval survivorship in northeast Texas. Direct effects of RIFA on monarch survivorship are measured by the correlated effects of RIFA abundance on monarch survivorship and the effects of manipulating RIFA abundance on monarch survivorship. Indirect effects are measured using an information criteria approach to examine the effects of RIFA abundance, host plant quality, and arthropod community dynamics on and around the host plant as they relate to monarch egg and larval survivorship. These latter relationships are compared for control plants and for plants where the abundance of RIFA have been manipulated. Collectively, these data should provide valuable information regarding how arthropod community dynamics, and RIFA in particular, affect monarch survivorship. Armed with this information, land managers can develop management strategies that optimize monarch egg and larval survivorship, thereby increasing local productivity.

The bulk of the research reported here was funded under a contract (#17-6192) with the Texas Comptroller's Office of Public Accounts, Economic Growth and Endangered Species Management Division. That contract, granted in 2017, followed a pilot study conducted in the spring of 2016 (Kopachena 2016, unpublished). This report includes some of the control data from the 2016 pilot study but focusses mainly on the more comprehensive data collected in 2017 and 2018. Both studies specifically target analyses of the survival of spring generation (Generation 1) monarchs in Texas. However, monarchs in north Texas also have a fall generation (Generation 5) for which there is very little information. Since information about the survivorship of fall monarchs may be important to the overall management of monarch butterflies in Texas, preliminary results from an independent study of fall survivorship of eggs and larvae in northeast Texas are also included in this report.

Methods

Data on monarch egg and larval survival in the springs of 2016 through 2018 were collected at the Cooper Lake Wildlife Management Area and adjacent portions of Cooper Lake State Park in Hopkins Co., Texas (33°18'51.09"N, 95°36'16.70"W) (Figure 1, 2, and 3). In the spring of 2016 data were collected from 28 March 2016 through 14 May 2016. In the spring 2017 data were collected from 21 March 2017 through 17 May 2017. In 2018 data were collected from 26 March 2018 through 11 May 2018.

The site was chosen because of the abundance of milkweed plants, the presence of RIFA, and ease of access. The density of RIFA mounds was measured as 617 mounds per ha (250 mounds per acre) in 2017 and 528 mounds per ha (213 mounds per acre) in 2018. The only milkweed species present was Green-flowered Milkweed (*Asclepias viridis*) with the exception of a small cluster of Butterfly Weed (*Asclepias tuberosa*) that was not included in the study. The density of milkweed plants, estimated in 2017, was 6540 plants per ha. The study area consisted of about 48 ha of open fields vegetated with native and exotic grasses and forbs along with wooded mottes and woodland margins. The northern portion of the study area was burned in the winters of 2004, 2007, 2011, 2013, 2016, 2017, and 2018.

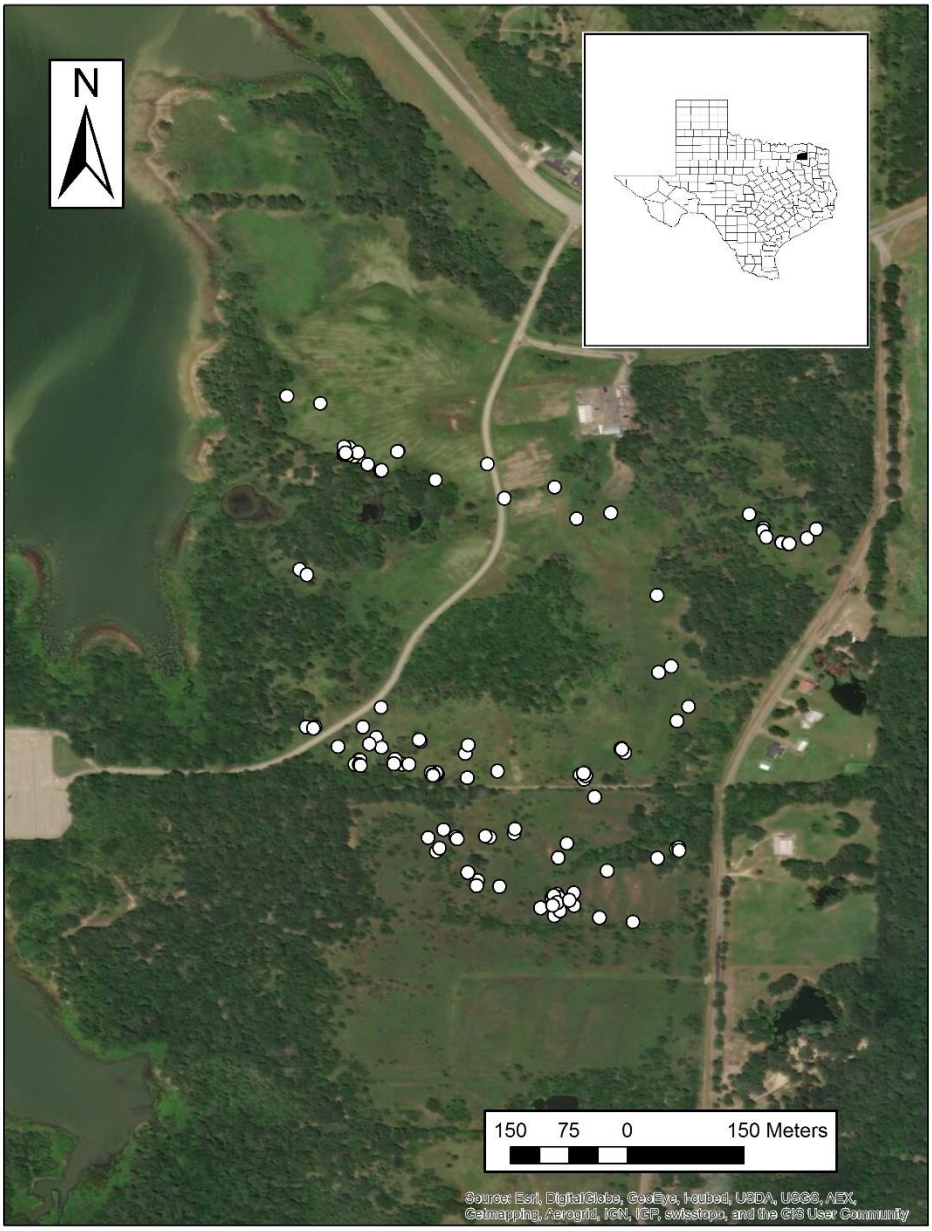

Figure 1. Study area in northeast Texas showing the locations of 122 host plants containing 215 eggs found in 2016. Inset map shows the location of the study area in relation to the state of Texas. This map shows only the locations of the control plants referred to in the body of this report.

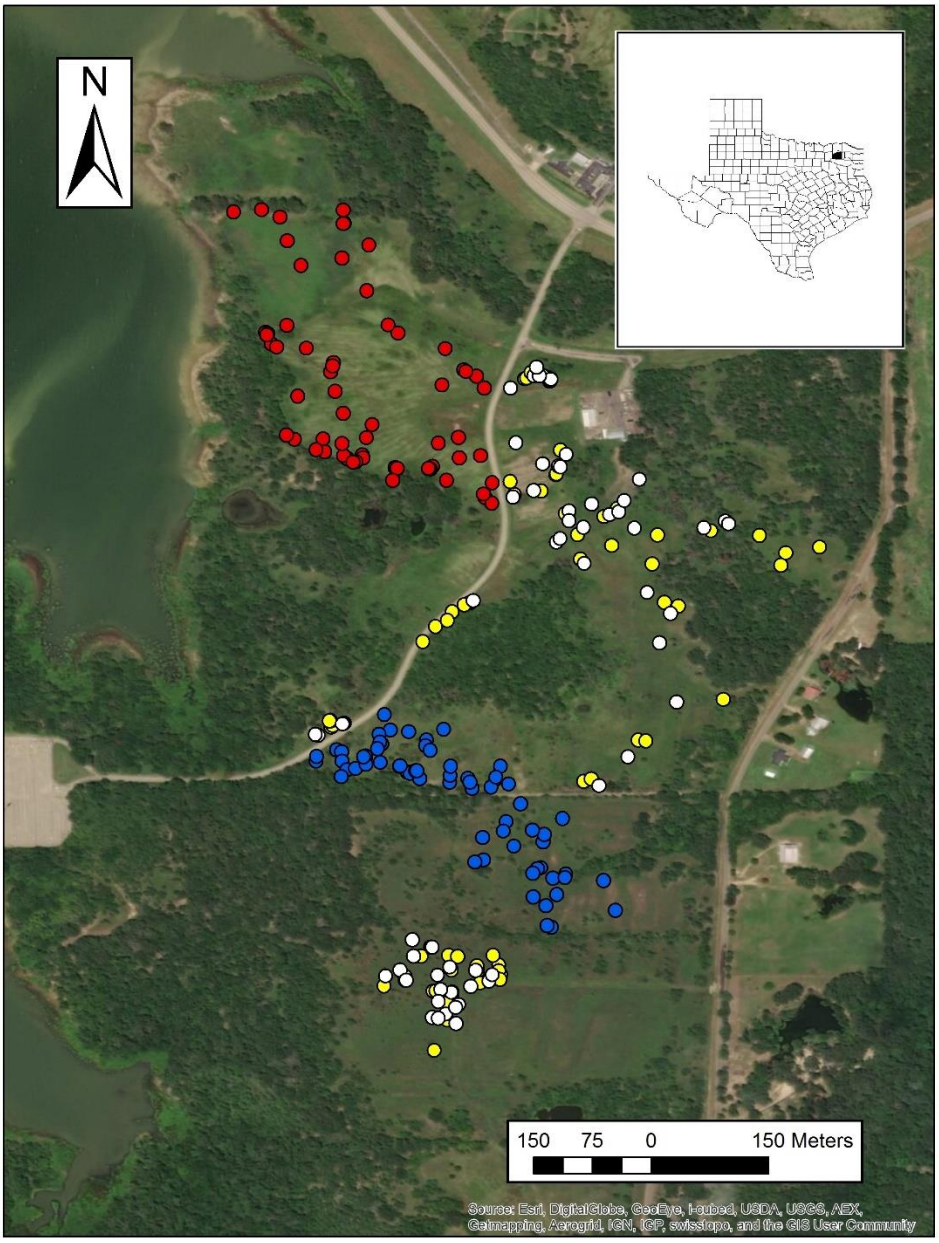

Figure 2. Study area in northeast Texas showing the locations of 262 host plants containing 416 eggs found in 2017. Inset map shows the location of the study area in relation to the state of Texas. Yellow circles are control plants without traps, white circles are control plants with traps, red circles are RIFA enhanced host plants, and blue circles are RIFA suppressed host plants. See methods of descriptions of treatments.

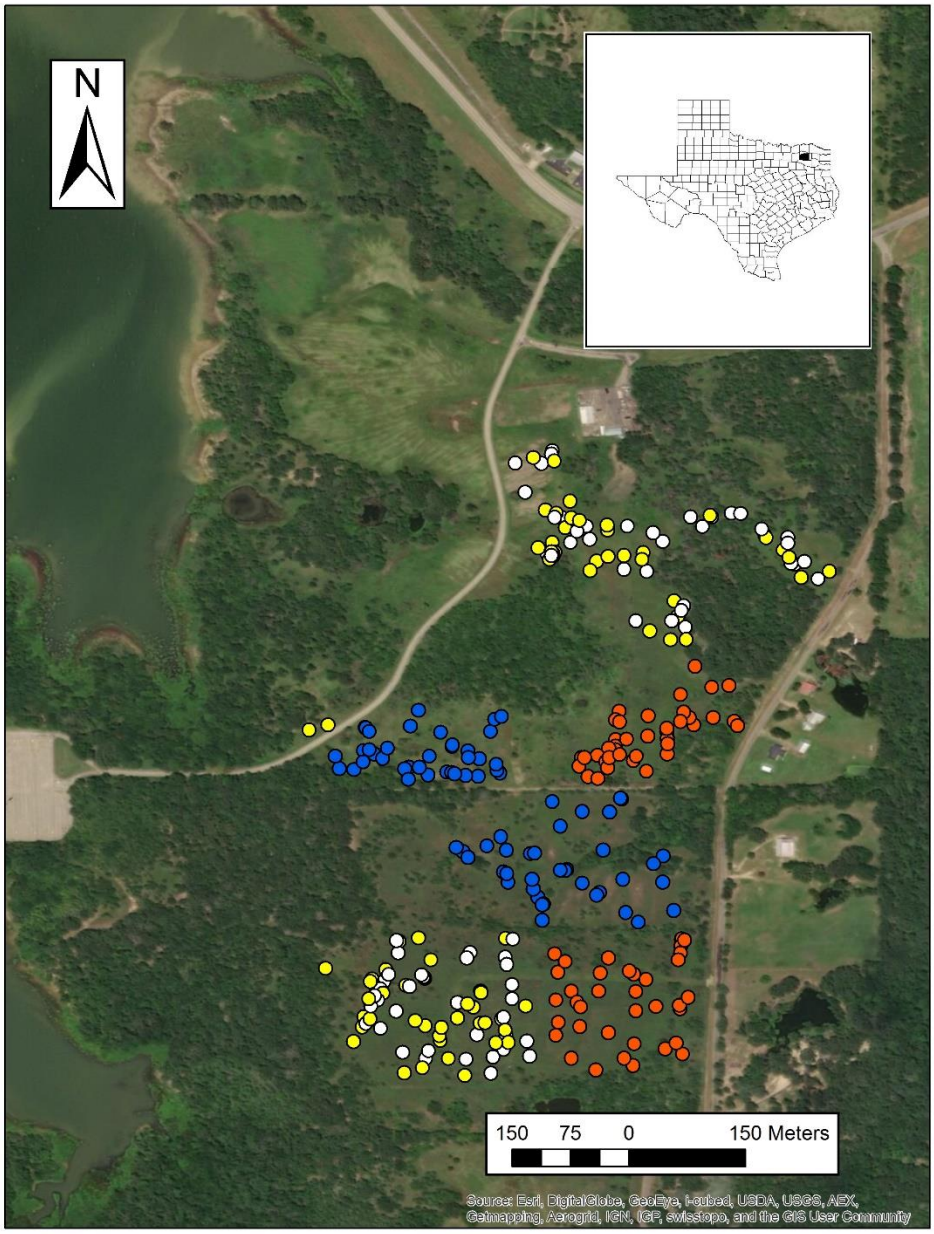

Figure 3. Study area in northeast Texas showing the locations of 301 host plants containing 503 eggs found in 2018. Inset map shows the location of the study area in relation to the state of Texas. Yellow circles are control plants without traps, white circles are control plants with traps, red circles are RIFA enhanced host plants, and blue circles are RIFA suppressed host plants. See methods of descriptions of treatments.

The southern portion of the study area had not been burned for over 20 years until the winter of 2018.

Monarch eggs were located by searching milkweed plants for eggs that had already been laid or by watching females oviposit on host plants. Once an egg was located the host plant was marked using a numbered flag (Figure 4). It was found that multiple eggs frequently occurred on the same plant. To keep track of individual eggs, the leaves containing the eggs were marked with numbers using a permanent felt-tipped marker. These marks had no impact, positive or negative, on the leaf, the eggs, or the instars. After heavy rains the marks tended to fade and sometimes had to be re-marked.

Plants with eggs or larvae were monitored every day between 10:00 h and 17:00 h. The pilot study (Kopachena 2016, unpublished) found that monarch larvae begin to emigrate off the host plants after they reach the third instar making mortalities difficult to document for older instars. Therefore, for this study, survivorship is measured as the number of individual reaching the third instar. Larvae, particularly first instars, can be difficult to find on the host plants and monarch larvae are known to temporarily leave the host plants for various reasons (Rawlins and Lederhouse 1981, Borkin 1982). The pilot study conducted in 2016 found that 86% of instars that were missing on one day were found again on the same plant within two days. Therefore, to ensure that a larva was not simply overlooked or temporarily off the host plant, once an egg or larva was missing, the host plant was visited for four more days. On the fourth day, if there was still no sign of the larva, observations were terminated for that host plant. Following the protocols of Zalucki and Kitching 1982, Zalucki and Brower 1992, and Prysby 2004, missing eggs or larvae were considered mortalities.

In 2017 and 2018, to test for the effects of RIFA on monarch survival and to document potential community-level interactions, the host plants were divided into four treatments: controls with traps, controls without traps, RIFA enhanced, and RIFA suppressed (Figures 2 and 3). Table 1 provides information on these treatments and the type of data collected from them.

All treatments except controls without traps used glue traps to document the terrestrial arthropod community around the host plant. For each plant, eight Victor Tin Cat Glue Board® bait-less traps were placed around and within 60 cm of the base of the plant on the first day and the next to last day that the

Figure 4. A host plant marked with a red flag. Eight glue traps were placed in a radial pattern around the plant on the first and second to last days that an egg or larva was monitored.

Table 1. Treatments used in this study and the types of data collected from each treatment.

Treatment	Protocol	Glue Traps	RIFA Abundance	Daily Host Plant Arthropods	Host Plant Condition	Cardenolides
Control no traps	Daily observations with no manipulations	X	✓	✓	✓	✓
Control with traps	Daily observations with no manipulations	✓	✓	✓	✓	✓
RIFA enhanced	Mealworms glued to bottom four leaves of plant	✓	✓	✓	✓	X
RIFA suppressed	Broadcast RIFA bait, individual mound treatments	✓	✓	✓	✓	✓

plant was monitored (Figure 4). For host plants that had multiple staggered eggs or larvae, glue traps were placed on the first and next to last day for each egg or larva on the plant. In cases where monitoring began or ended a day apart for two or more eggs or larvae, data from the same date were used for each egg or larva. Glue traps were arranged in a radial pattern around the host plant and landscaping pins were used to hold the traps firmly and flush with the soil surface (Figure 4). The traps were left out for 24 h after which all of the arthropods in each trap were identified and counted. To test for the effects that placing glue traps might have on monarch mortality and on plant arthropod populations, half of the control plants did not have traps set next to them (controls without traps). Both control treatments occurred in the same portions of the study area (Figures 2 and 3), though different types of controls were always separated by at least 3 m.

The RIFA enhanced treatment was created by gluing dried mealworms onto the lower four leaves of a host plant (Figure 5), thereby drawing RIFA onto the host plants. Elmer's Wood Glue® was used as a non-toxic adhesive for this purpose. The mealworms were quickly consumed by RIFA and, during rainy weather, sometimes washed off the leaves, so worms were replenished daily to keep the RIFA on the host plant. To avoid affecting host plants in other treatments, RIFA enhanced plants were in a separate portion of the study site and separated from other treatments either by roadways and easements or by areas devoid of host plants (Figures 2 and 3). In 2017, the RIFA enhanced treatments were confined to the northwestern portion of the study area (Figure 2). However, due to low densities of RIFA in this area, in 2018 the RIFA enhanced treatments were moved to two smaller areas that were closer to the other treatments (Figure 3).

The RIFA suppressed treatment was created by broadcasting RIFA bait and individual mound treatments prior to the onset of the field season and, occasionally, by individual mound treatments during the field season. To avoid affecting host plants in other treatments, the RIFA suppressed treatment was limited to one portion of the study area and separated from other treatments by roadways, easements, and wooded areas (Figures 2 and 3). The RIFA bait used in this study was Extinguish Plus Fire Ant Bait® which is composed of Hydramethylnon 0.365% and S-Methoprene 0.250% in a corn meal carrier. This

Figure 5. RIFA attacking a mealworm that has been glued to a milkweed leaf. For RIFA enhanced treatments, a dried mealworm was attached to each of the bottom four leaves of the host plant using a non-toxic wood glue. Mealworms were replaced when consumed or if they fell off the plant.

broadcast bait targets RIFA with minimal impacts on non-target invertebrates (Drees et al. 2013) and has been used in other studies that examined the impact of RIFA on arthropod communities (Eubanks et al. 2002). The bait was applied at the recommended application rate of 2.5 lbs/acre. Broadcasting of RIFA bait occurred three times prior to the onset of the 2017 spring field season on 24 and 25 October 2016, 7 and 8 March 2017, and 20 March 2017. Broadcast baiting of RIFA was then repeated during the following summer, fall, and early spring on 27 June 2017, 12 October 2017, 3 March 2018, and on 20 March 2018. At these times, individual mounds were also treated with the same bait.

No RIFA bait was broadcast once the field season began on 21 March 2017 and on 26 March 2018. However, as each field season advanced, new RIFA mounds would occasionally appear on the treated area. When this occurred, the new mounds were treated with Bayer Advanced Fire Ant Killer Dust® which contains 0.5% β-Cyfluthrin. β-Cyfluthrin is sensitive to sunlight and exposed treatments have a half-life of 48 to 72 hours (Cyfluthrin, EXTOXNET, Cornell University, 1995). This treatment typically killed the ants within 24h and care was taken to avoid exposure of the powder to surfaces other than the RIFA mound.

In 2016, only the distance from the host plant to the nearest RIFA mound was measured. In 2017 and 2018, RIFA abundance was measured as the number of active mounds within 4 m of the host plant, the volume of active mounds within 4 m of the host plant, and the distance of the host plant to the nearest active mound. The volume of mounds was based on the portion visible above the ground and was calculated as ½ the volume of an ellipsoid using the mound length, width, and height to estimate the principle axes. The total volume of all fire ant mounds within 4 m of the host plant and was measured in cm^3. RIFA abundance was measured on the day after an egg or larva was found and on the last day that the egg or larva was monitored. For statistical analyses, the average of the two days was used.

Host plants in all treatments were visited every day to monitor monarch egg and larval presence on the plant. In addition, in 2017 and 2018, each day that the plant was visited, all other arthropods on the plants were noted and counted. These data were later compiled and used to look for community-level interactions on the host plants.

To determine the extent that host plant condition might affect monarch egg and larval survival, data were also collected on the physical condition and appearance of the host plants in 2017 and 2018. Like the trap data, the data on host plant condition were collected twice for each plant; on the day after an egg was found and on the last day that the host plant was monitored. Physical characteristics of the host plant quantified were the number of ramets, the length of each ramet, the number of adult leaves on each ramet, leaf curling, general necrosis of leaves, wilting of leaves, darkening of leaf veins, general darkening of leaf blades, yellowing of leaf blades, extent of leaf spotting, shoot tip necrosis and wilt, and herbivory. A general description of these traits and how they were quantified follows.

For each host plant, the ramets were identified as all the stems radiating from a central point in the ground. The length of each ramet was measured from its base to the shoot tip. The number of fully unfurled leaves was then counted for each ramet. The number of ramets, the total length of the ramets, and the total number of leaves on all ramets were used to evaluate the size of the host plant.

Normal leaves for *A. viridis* are ovate to lanceolate and have blades that are light green with pale venation (Figure 6A, Figure 7A). The leaves are typically rather flat with slightly upturned margins. Frequently, however, the leaves exhibit varying levels of longitudinal curling possibly in response to stress (e.g. Figure 6B). To quantify leaf curling, standard area diagrams with a five-point scoring scale were developed (Figure 6C). For each ramet on each plant, every adult leaf was scored and the average score of the ramet was then calculated. These averages were then used to calculate an overall weighted average curling score for the whole plant.

A. viridis leaves also exhibit a variety of other morphological and pathological variations (Figure 7). Some plants exhibited deep purple veins (Figure 7B). Many plants exhibited dark spotting on the leaves (Figure 7C). Some plants exhibited discoloration not involving the leaf veins, either in the form of darkening (Figure 7D) or yellowing (not illustrated). Lastly, some plants were infected with leaf miners (Diptera: Agromyzidae) (Figure 7E).

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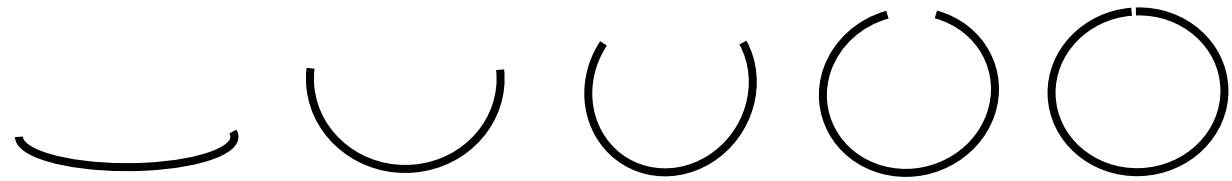

Score 1	Score 3	Score 5	Score 7	Score 9
Leaf margins at <90° from leaf bottom	Leaf margins about 90° from leaf base	Leaf margins more than 90° from leaf base but not nearly occluded	Leaf margins more than 90° from leaf base and nearly occluded	Leaf margins occluded

Figure 6. Leaf curling in A. viridis and standard area diagrams used to score leaf curling. **A.** Typical leaf showing very little curling, scored as 1. **B.** A leaf whose curling has completely occluded and would be scored as 9. **C.** Standard areas diagrams with descriptions and scoring system.

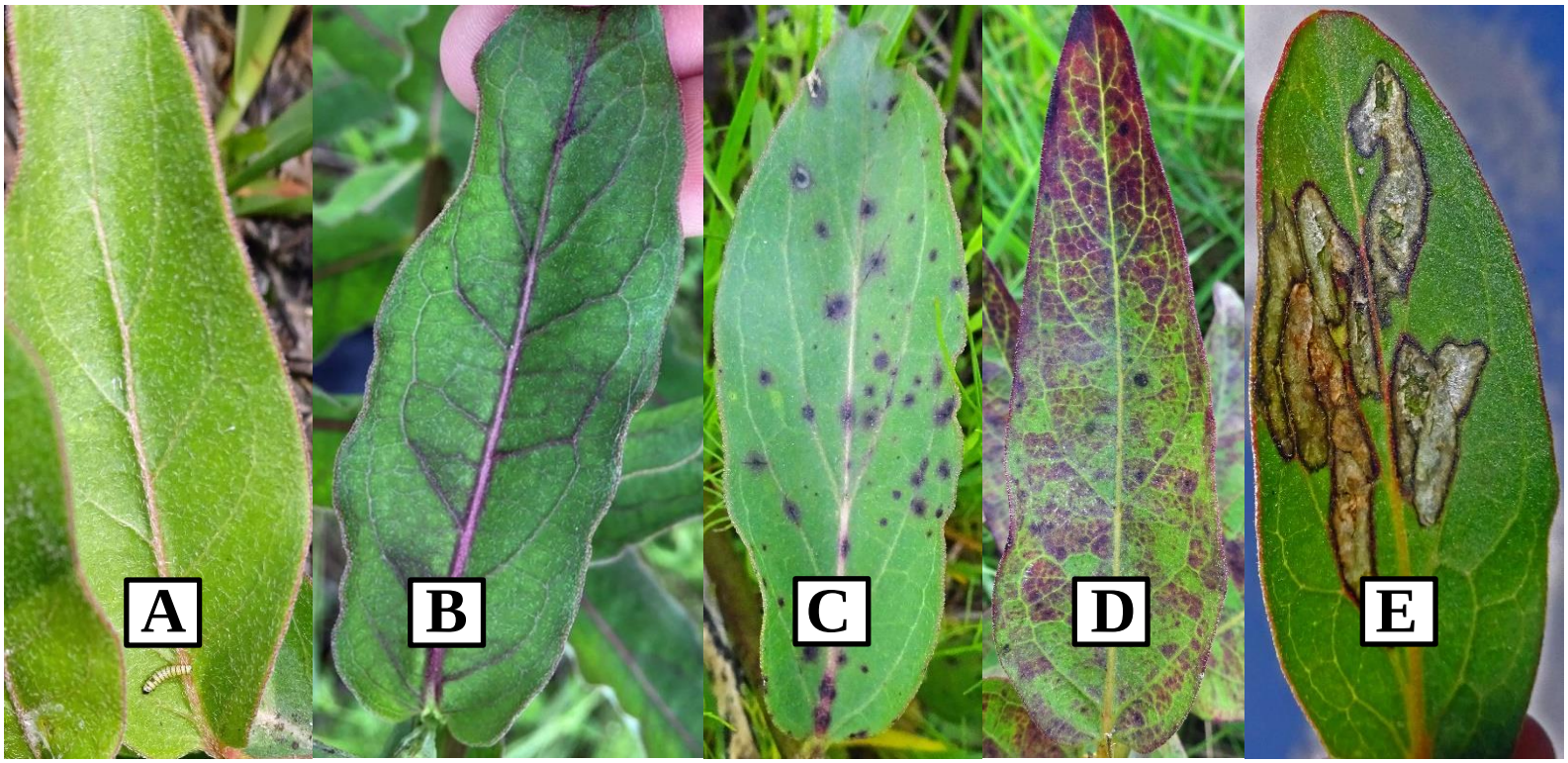

Figure 7. Morphological and pathological traits exhibited by *A. viridis*. **A.** A normal, healthy leaf. **B.** A leaf showing deep purple pigmentation associated with the leaf veins. **C.** A leaf exhibiting leaf spots. **D.** Dark blotching not involving veins. **E.** Leaf Miner (Diptera: Agromyzidae) damage.

To facilitate analyses of these traits, for each ramet on each plant, the number of affected leaves was recorded and a photograph was taken of a representative leaf exhibiting each trait. Compu Eye Leaf Symptom Area software (Bakr 2005) was used to quantify the extent of coverage of each trait on the photographed leaf (Figure 8). The percent coverage of the leaf was then multiplied by the total number of affected leaves on the ramet and the sum of these values for all ramets was divided by the total number of leaves on the plant to create a weighted index of trait intensity.

There is no published information on pathologies and diseases of *A. viridis*. In order to make more informed evaluations on the effect of plant health on monarch egg and larval survival, soil samples and plant samples were sent for analyses of soil chemistry and plant pathogens. Ten plants were selected for each of the following characteristics: normal leaves and growth form, purple veins, leaf spots, darkening not involving the leaf veins, and yellowing of leaf material. For each of the ten plants exhibiting each symptom, soil samples were collected within 50 cm of the base of the plant. Soil samples were collected by clearing the surface of the soil of organic debris and collecting 500 cm^3 of soil from the surface to a depth of approximately 20 cm. These samples were sent to the Soil, Plant & Water Analysis Laboratory at Stephen F. Austin State University in Nacogdoches, Texas. These samples were analyzed for phosphorous, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, copper, nitrate, electrical conductivity, and pH. To evaluate the presence of plant pathogens, for each of the ten plants exhibiting each of the symptoms, the entire plant, including the roots, were sent to the Texas Plant Disease Diagnostic Lab at Texas A&M University, College Station, Texas. These plants were screened for common plant fungal, bacterial, and generalist viral diseases including Potyvirus, Impatiens Necrotic Spot Virus, Tomato Spotted Wilt Virus, and Cucumber Mosaic Virus.

Some plants, typically those infested with stem weevils, exhibited shoot tip wilting and necrosis (Figure 9). For these plants, a series of reference images were used to develop a disease assessment key with a five-point scoring scale (Figure 9).

Data were also collected on the number of leaves exhibiting general necrosis, herbivory, and general wilt (defined as a visible loss of turgor). For these traits an index of intensity was calculated as

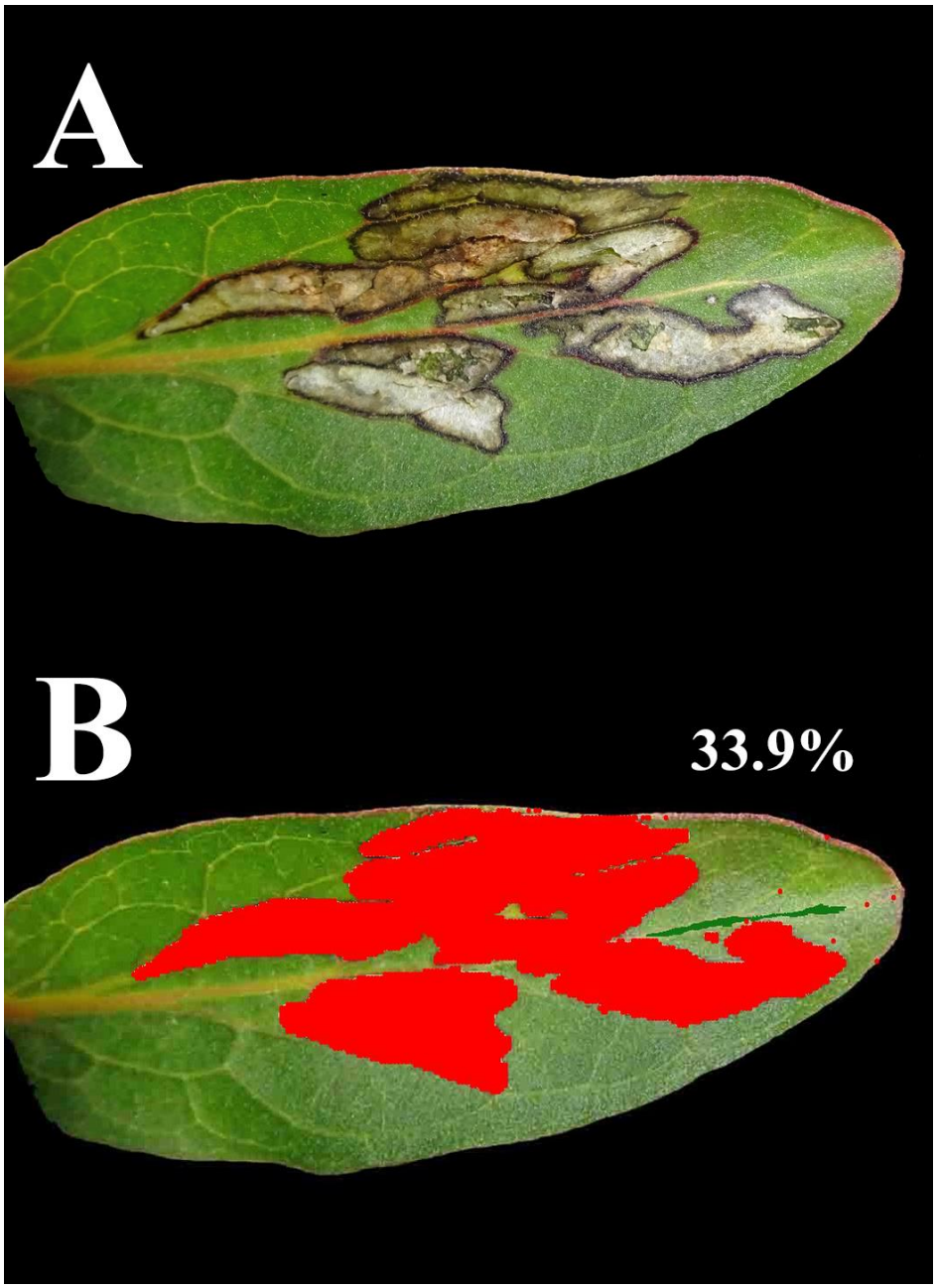

Figure 8. Method used to analyze leaf symptom area using Compu Eye Leaf Symptom Area software (Bakr 2005). **A.** A photograph of a leaf, in this case showing leaf miner damage, is isolated and a black background is put on the image. **B.** The software scans the image and, based on user inputted criteria, quantifies the affected area. In this example, 33.9% of the leaf area is affected.

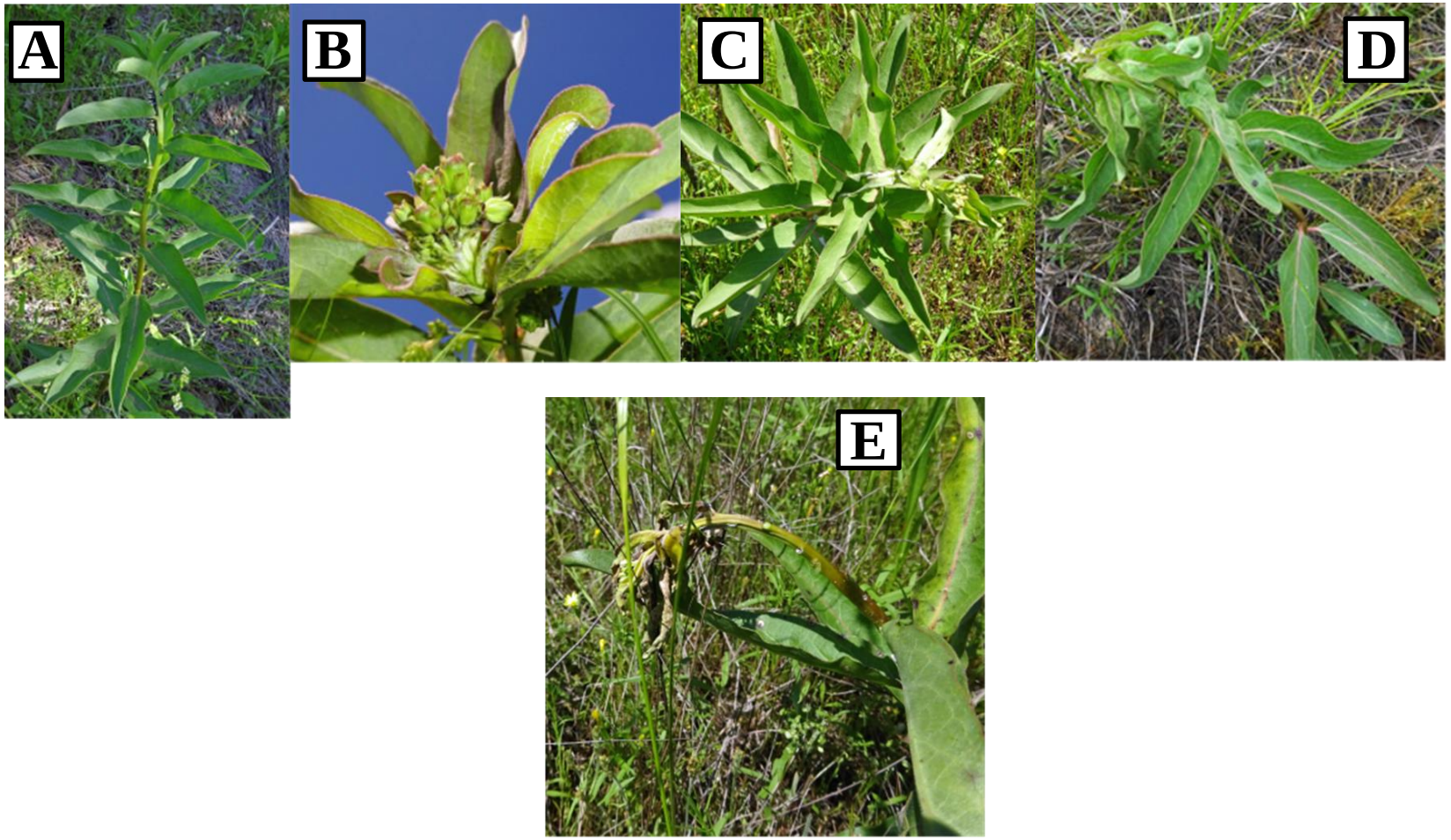

Figure 9. Shoot tip necrosis and wilt caused by stem weevils in *A. viridis*. **A.** Normal plant showing no pathology to shoot tip. Score = 1. **B.** Very slight disfiguring of terminal leaves only. Score =3. **C.** Shoot tip clearly wilted, leaves contorted and pale, but limited to upper ¼ of plant. Score = 5. **D.** Shoot tip strongly wilted, leaves are contorted and pale, extending beyond the upper ¼ of plant. Score = 7. **E.** Shoot tip necrotic and strongly wilted, leaves contorted and pale, extending beyond the upper ¼ of plant. Leaves may be missing or falling off. Score = 9.

the number of affected leaves on all ramets divided by the total number of leaves on the plant. Likewise, for each ramet on each plant, the number of stem weevil oviposition sites was counted and an index of stem weevil infestation was defined as the total number of oviposition holes divided by the total length of ramets on the plant. In addition, a few plants were browsed by rabbits and hares (mainly eastern cottontails, *Sylvilagus floridanus*) and these instances were also recorded.

The last attribute of plant condition measured was leaf total cardenolides. On the last day that each plant was monitored a sample of three adult leaves were collected from the host plant, one from the lower part of the plant, one from the middle portion of the plant, and one near the top of the plant. A similar set of three leaves were collected from an adjacent, apparently unoccupied, *A. viridis* plant if a comparable plant was present within 2m of the host plant. The leaf samples were placed in a cooler and transported to the lab where they were stored at -80^{o} C. Total cardenolide concentrations were quantified using reflectance spectroscopy following the method of Couture et al. 2013. Prior to analysis the samples were air dried at 50^{o} C for 24 h and cardenolides were extracted in 95% ethanol. The absorbance of samples was compared to a digitoxin standard curve to measure total cardenolides in mg/0.1 g.

There is little data on the survivorship of monarch eggs and larvae in the fall in Texas. Therefore, in addition to the spring study described above, a simpler study was conducted on fall monarch egg and larval survival. In the fall of 2017, from 15 August until 26 October, 250 eggs and larvae on 207 host plants were identified on a 27.5 ha tract of land adjacent to the city of Sulphur Springs, Texas (Figure 10). This study site was located 19 km due south of the spring study site. A separate study site had to be used because the spring site is not mowed or burned during the summer and fall and, as a result, most of the milkweed on that site had senesced by late summer and fall. The fall study site is mowed on a regular basis and, in 2017, was mowed at the end of June.

Following the same protocol as in the spring study, monarch eggs were located by searching potential host plants. Once found, the leaves upon which the eggs occurred were marked with a non-toxic marker, the host plant was marked with a white flag, and the host plants were visited daily until the egg or larva either went missing for four days or reached the third instar. For the fall data the number of RIFA

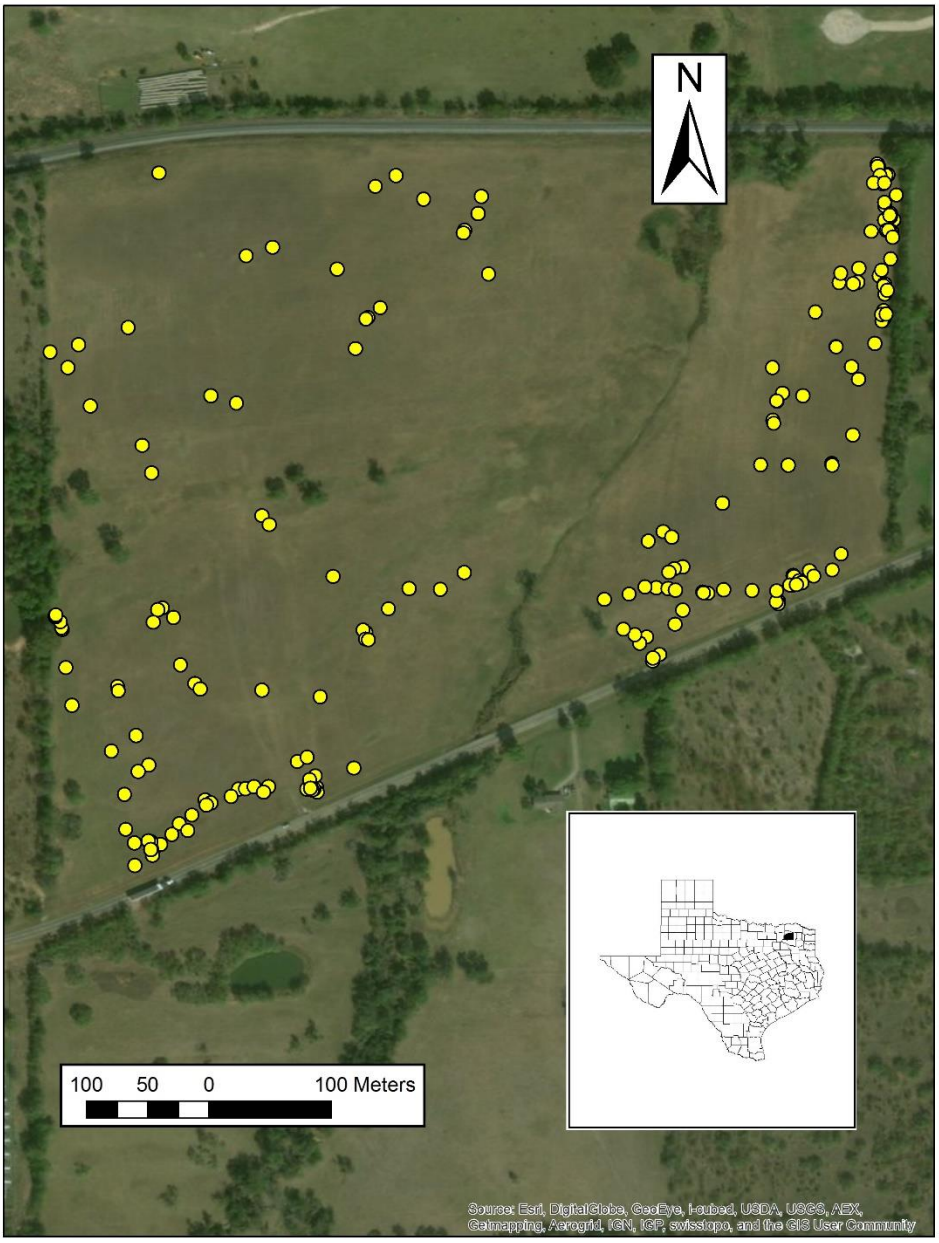

Figure 10. Study area in northeast Texas showing the locations of 207 host plants containing 250 eggs found in the fall of 2017. Inset map shows the location of the study area in relation to the state of Texas.

mounds within 4m of the host plant were counted and all of the arthropods on the host plant were counted and recorded.

Finally, meteorological records were collected for each year and each study area from the NOAA (National Oceanic and Atmospheric Administration) National Centers for Environmental Information weather monitoring stations (https://www.ncdc.noaa.gov/cdo-web/). For the spring study site daily high and low temperatures and monthly precipitation totals were available for a monitoring station located 0.6 km from the center of the study site. However, long-term averages for temperature and precipitation were only available from a monitoring station in Sulphur Springs, Texas, located 17 km. from the center of the spring study site. Long-term averages were based on data collected from 1981 through 2010. For the fall study site, all weather data was based on the Sulphur Springs monitoring station which was located 3.7 km from the center of the study site.

All statistical analyses were conducted using SAS software (SAS Release: 3.7 (Enterprise Edition) © 2012-2017, SAS Institute Inc., Cary, NC, USA).

Results

The data used in this report consist only of individuals first found as eggs. In 2016, this was further limited only to control plants. For 2016, there were 215 eggs on 122 host plants (Figure 1). These data were used only for analyses of survivorship and the relationship of survivorship to the nearest RIFA mound. All other analyses are based only on the 2017 and 2018 data.

For 2017, data were collected for 384 eggs on 260 host plants. These were divided into 95 eggs on 65 control host plants with no traps, 97 eggs on 65 control host plants with traps, 85 eggs on 65 RIFA enhanced host plants, and 107 eggs on 65 RIFA suppressed host plants (see Figure 2). In 2018, 503 eggs were found on 301 host plants and these were divided into 130 eggs on 77 control host plants with no traps, 127 eggs on 73 control host plants with traps, 120 eggs on 77 RIFA enhanced host plants, and 126 eggs on 74 RIFA suppressed host plants (see Figure 3).

As a preliminary analysis, for the 2017 data, the effect of flag color on survival and number of individuals on a plant was tested. None of these parameters varied relative to flag color (Survival, X^2 = 0.7767, df = 3, p = 0.8550; Number of Individuals per Plant, X^2 = 9.7683, df = 6, p = 0.1348). For this reason, all the data for the various flag colors were combined for further analyses.

a. Phenological and Meteorological Considerations

In north Texas, monarchs begin to arrive toward the end of March and continue to lay eggs until at least the end of April. Long term average temperatures, based on data collected from 1981 through 2010 (NOAA National Centers for Environmental Information, Sulphur Springs, Texas, weather monitoring station (https://www.ncdc.noaa.gov/cdo-web/)), show that temperatures gradually increase across this time period. Average low temperatures climb from 6.7° C in mid-March to 16.1° C in mid-May. Average high temperatures increase from 18.0° C in mid-March to 26.7° C in mid-May. However, temperatures can vary considerably from year to year and the period from 2016 through 2018 was no exception (Figure 11). April daily low temperatures were above average for 2016 and well below average for 2018. Daily high temperatures in April were well above average for both 2016 and 2017 (Figure 11).

There were also strong deviations from normal precipitation across years (Figure 12). In particular, April precipitation was almost four times higher than average in 2016 and more than twice the average in 2017. In contrast, April precipitation in 2018 was only about 40% of the average precipitation expected for the month of April (Figure 12).

Differences in weather conditions among years was likely responsible for differences in the phenology of vegetation and arthropod emergences among years. These differences were documented for 2017 and 2018 (Figure 13). During the cooler, drier year of 2018, milkweed flowers opened 17 days later and first instars appeared 5 days later than they did in the warmer, wetter year of 2017 (Figure 13). Milkweed bugs (*Oncopeltus fasciatus*), weevils, (Curculionidae), and monarch instars also appeared later in 2018 than in 2017. There were differences in the order in which some arthropods appeared. For example, in 2018, weevils appeared before milkweed bugs, whereas the opposite was true in 2017.

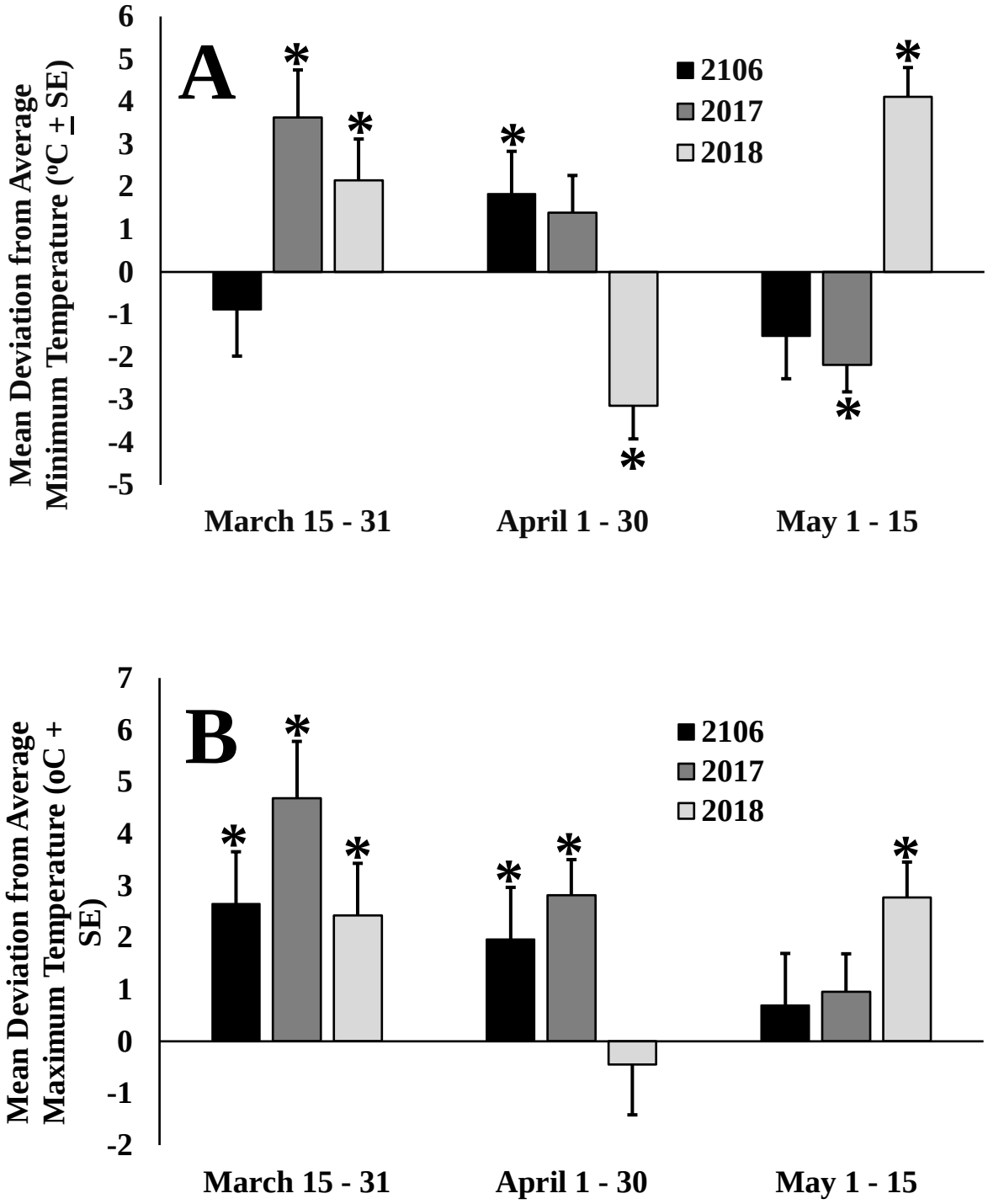

Figure 11. Deviation of daily temperatures from normal for the years 2016 through 2018. Normal temperatures based on data collected from 1981 through 2010 (NOAA National Centers for Environmental Information for Sulphur Springs, Texas (https://www.ncdc.noaa.gov/cdo-web/)). Asterisks indicate temperature deviations that were significantly different from normal (ANOVAs, $p < 0.05$). A. Deviations from normal daily low temperatures. B. Deviations from normal daily high temperatures.

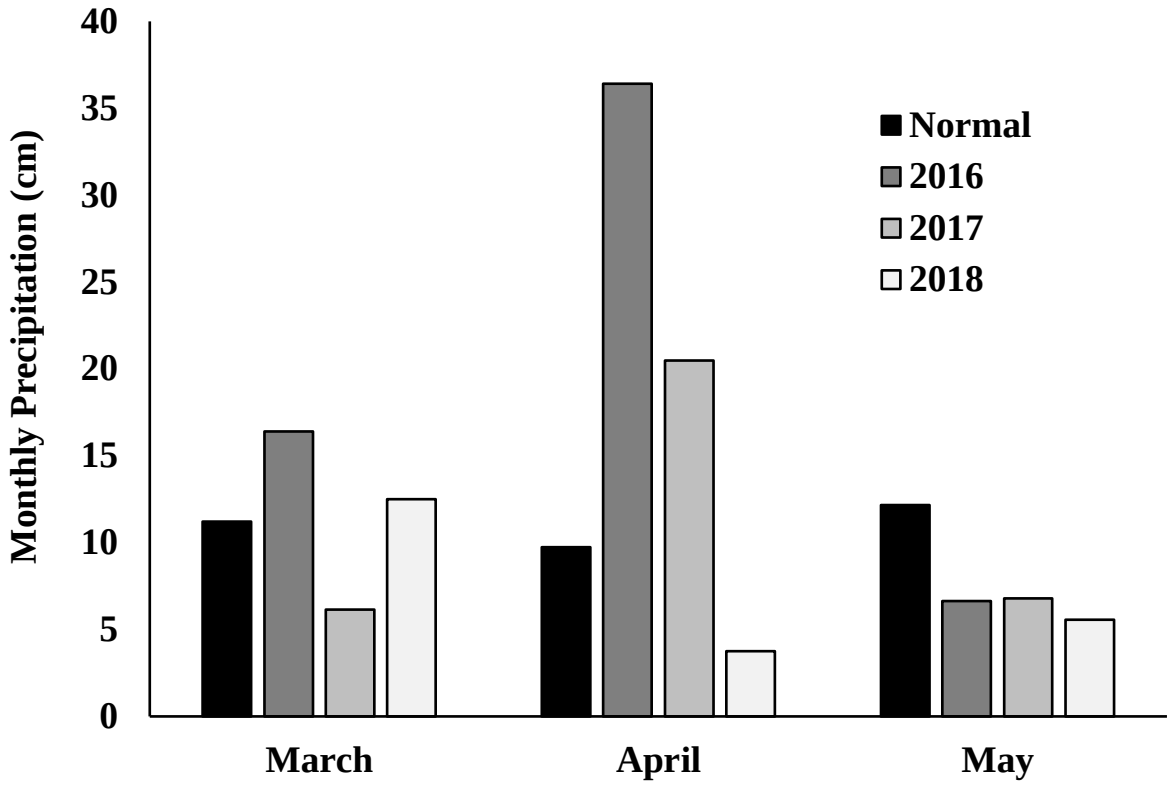

Figure 12. Precipitation as compared to normal for 2016 through 2018. Long-term normal precipitation based on Sulphur Springs recording station from 1981 through 2010. Data for 2016 through 2018 were recorded at Cooper Dam on Jim Chapman Reservoir and adjacent to the study site. All data were retrieved from NOAA (National Oceanic and Atmospheric Administration) National Centers for Environmental Information on 31 May 2018 (https://www.ncdc.noaa.gov/cdo-web/).

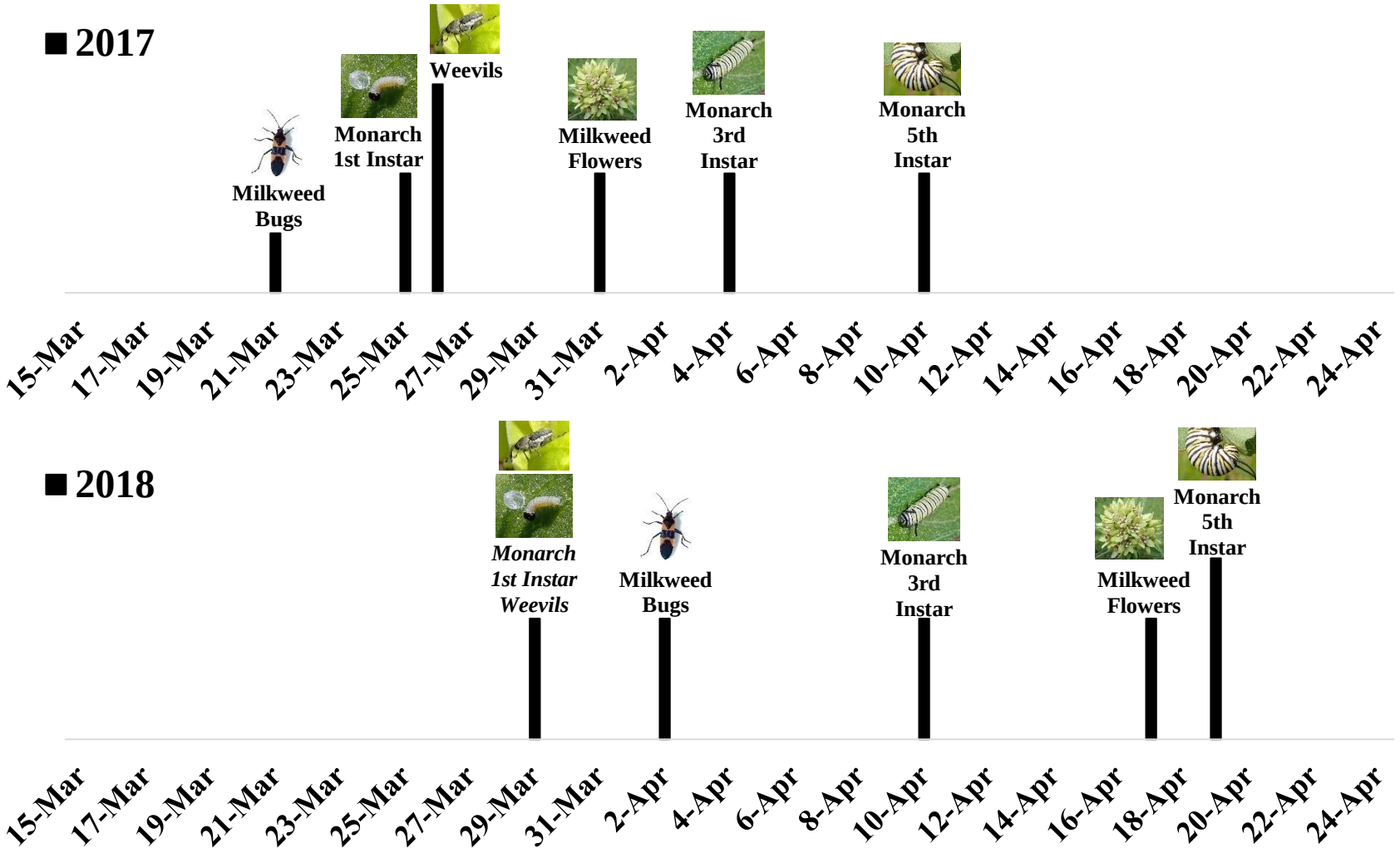

Figure 13. Phenology of monarch eggs and larvae, milkweed bugs, weevils, and milkweed inflorescences in 2017 and 2018.

Similarly, differences in temperature and precipitation among years may have affected the abundance and activity of RIFA (Figure 14). Based only on control data, there were no statistically significant differences among years in the distance of host plant from RIFA (Figure 14A). However, when comparing RIFA abundances associated with control treatments between 2017 and 2018, the cooler, drier year of 2018 was characterized by fewer mounds adjacent to host plants (Figure 14B), a lower overall volume of RIFA mounds adjacent to host plants (Figure 14C), and fewer RIFA captured in glue traps adjacent to host plants (Figure 14D). Despite this, there was no significant difference between years in the number of RIFA found on control plants (ANOVA, $F = 0.170$, $df = 1, 429$, $p = 0.6763$).

There was a tendency for daily monarch egg and larval survival rate, calculated as the proportion of individuals that did not go missing during any given 24h period, to decline with advancing date during the month of April. This was most pronounced in spring of 2018 (Pearson's $r = -0.577$, $p = 0.0007$, $n = 31$), but not statistically significant in spring of 2017 (Pearson's $r = -0.185$, $p = 0.3189$, $n = 31$).

b. Monarch Survivorship and RIFA abundance

For 2016 through 2018, RIFA abundance was measured as the distance to the nearest RIFA mound from the host plant. In 2017 and 2018, RIFA abundance was also quantified as the number of mounds within four meters of the host plant, the total volume of RIFA mounds within four meters of the host plant, and the number of RIFA observed on the host plant. The number of RIFA on the host plant was calculated as the total number of RIFA observed on the plant divided by the number of days that the plant was under observation. Finally, there were counts of RIFA captured in glue traps adjacent to the host plants.

Analyses were run to evaluate the effectiveness of the baiting and mound treatments used to suppress RIFA adjacent to host plants. There were markedly fewer RIFA in the suppressed treatment as compared to controls (Figure 15). In particular host plants in the RIFA suppressed treatment were much farther from the nearest RIFA mound (Figure 15A), had fewer RIFA mounds within 4.0 m of the plant (Figure 15B), had a lower volume of RIFA mounds within 4.0 m of host plants (Figure 15C), and were

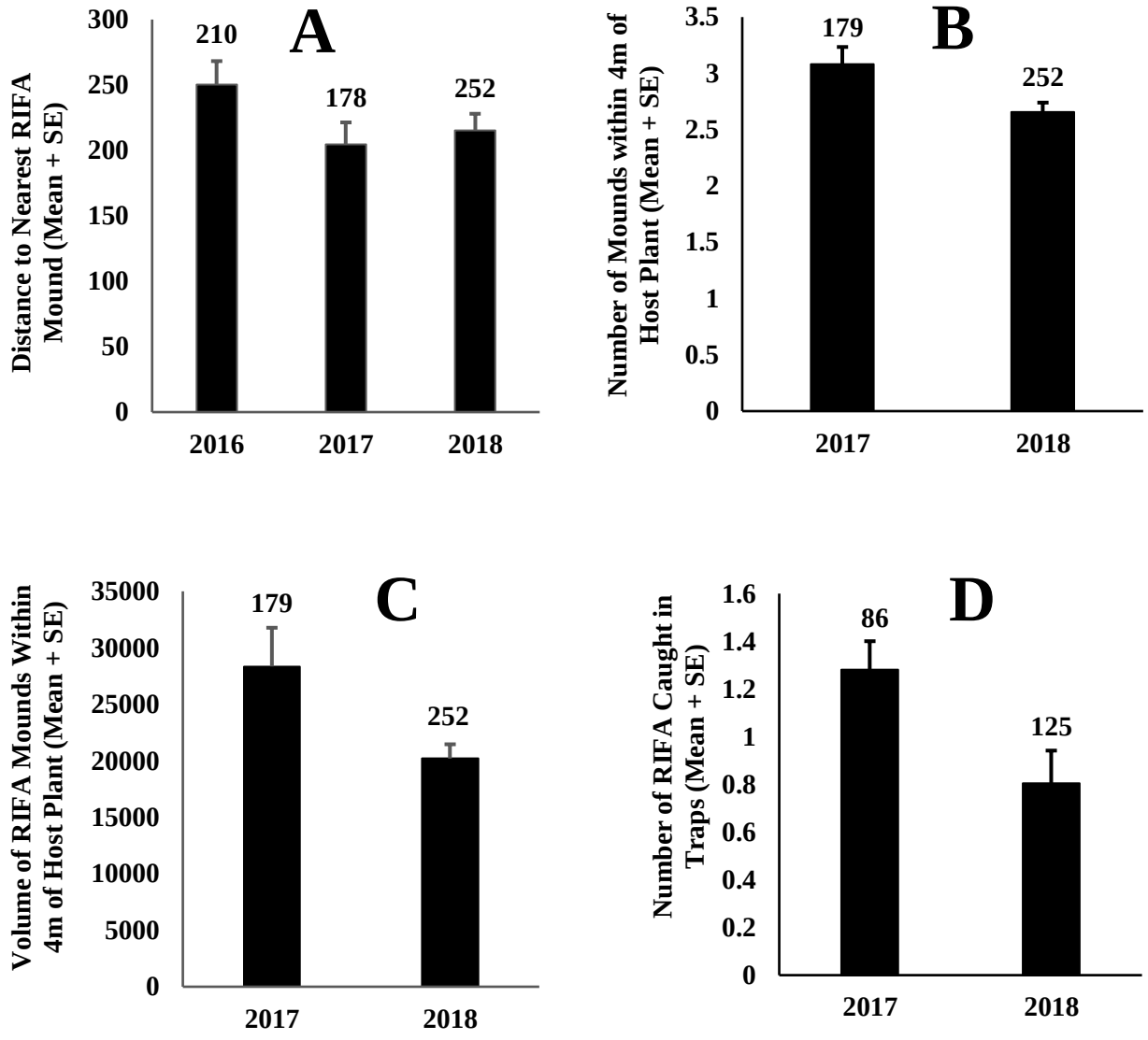

Figure 14. Annual variations in RIFA abundance adjacent to control host plants. Numbers above bars are sample sizes (number of monarch eggs). A. Distance of host plant to nearest RIFA mound. ANOVA, $F = 2.24$, $df = 2, 637$, $p = 0.1068$. B. Number of mounds within 4 m of host plant. ANOVA, $F = 7.00$, $df = 1, 429$, $p = 0.0085$. C. Total volume of RIFA mounds within 4 m of host plant. ANOVA, $F = 6.628$, $df = 1, 429$, $p = 0.0126$. D. Number of RIFA captured in glue traps adjacent to host plants. ANOVA, $F = 6.15$, $df = 1, 209$, $p = 0.0139$.

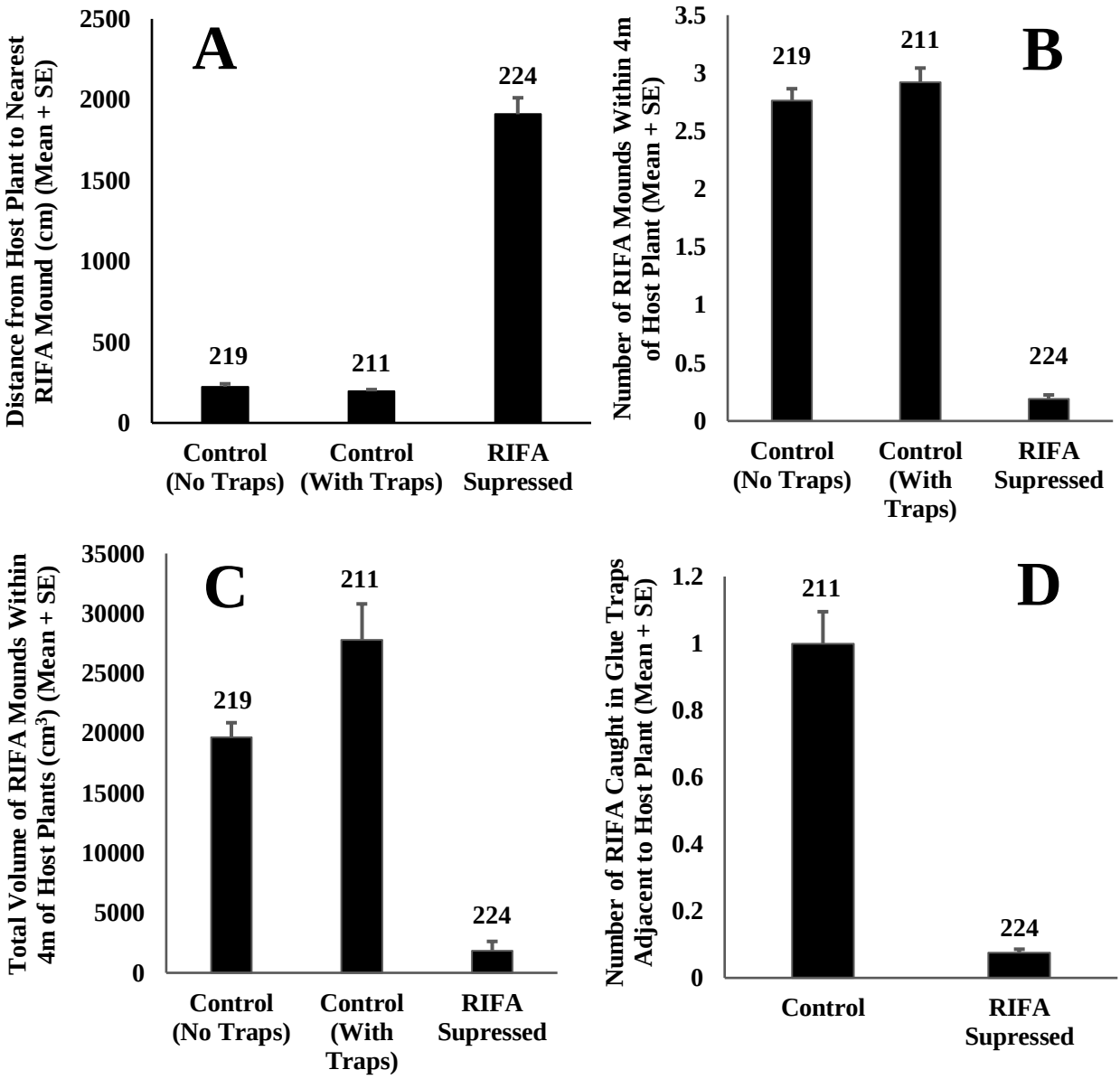

Figure 15. Efficacy of RIFA suppression relative to control treatments. Numbers over bars represent sample sizes (number of monarch eggs). A. Distance from host plant to nearest RIFA mound. ANOVA, $F = 256.44$, $df = 2, 651$, $p < 0.0001$. B. Number of RIFA mounds within 4.0 m of host plants. ANOVA, $F = 271.14$, $df = 2, 651$, $p < 0.0001$. C. Total volume of RIFA mounds (cm^3) within 4.0 m of host plants. ANOVA, $F = 49.68$, $df = 2, 651$, $p < 0.0001$. D. Number of RIFA captured in glue traps adjacent to host plants. ANOVA, $F = 97.58$, $df = 1, 433$, $p < 0.0001$.

associated with fewer captures of RIFA in glue traps adjacent to the host plant (Figure 15D). These data show that, though suppression did not entirely eliminate RIFA, RIFA abundance in the suppressed treatment was, by any measure, at least 10 times lower than for the controls.

Gluing mealworms onto the host plants effectively increased the number of RIFA on the host plants (Figure 16). Consequently, there were fewer RIFA on host plants in the RIFA suppressed area than were found on control plants and far more RIFA on plants in the enhanced treatment than were found in any other treatment group. In terms of frequencies, only 5 (2%) host plants had RIFA in the RIFA suppressed treatment, whereas all but one (99.5%) of the host plants in the RIFA enhanced treatment contained RIFA. Controls, with and without traps, had occupancy rates of 27% and 30% respectively.

Most of the eggs used in this study were of unknown age when they were found. Survival estimates based on individuals of unknown age are inflated because they favor of individuals that survive. This is because the sample does not include individuals that perished before they could be found. To correct for this, daily survivorship rates were calculated and multiplied by the known duration of the age class for which survival was being estimated (eggs in the current study). This is known as the Mayfield Method (Mayfield 1975, Greeney et al., 2010). In order to determine the length of time that monarch eggs take from laying to hatching, individuals of known age are required. That was obtained by observing female monarchs ovipositing and following these eggs until they hatched. In this way, in 2016, 20 eggs of known age were followed to hatching, in 2017, 16 eggs of known age were followed to hatching, and in 2018, 27 eggs of known age were followed to hatching. Monarch development is temperature dependent (Zalucki 1982) so the length of time to hatching varied among years, being 7.85 days in 2016, 6.56 days in 2017, and 8.19 days in 2018. These intervals were used to calculate the survival of eggs. Since the age of the instars included in the analyses was known, it was unnecessary to apply the Mayfield Method to first and second instars.

The Mayfield corrected survivorship does not allow for statistical comparisons because the data do not follow a known mathematical distribution. Therefore, statistical comparisons are based on the

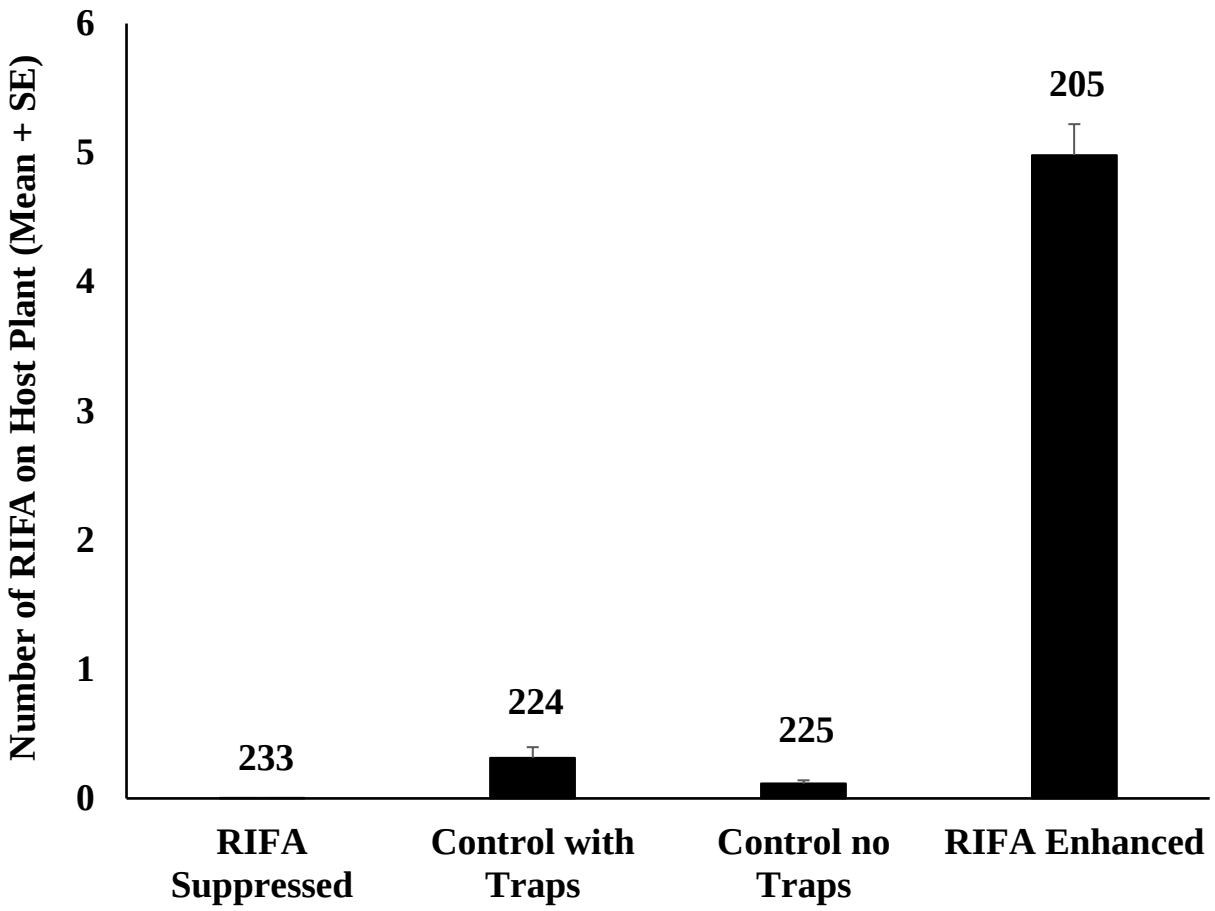

Figure 16. Number of RIFA on host plants relative to treatment. Numbers over bars represent sample sizes (number of monarch eggs). The number of RIFA on host plants was measured as the number of individuals per day of observation and differed markedly among treatments (ANOVA, $F = 392.41$, $df = 3, 883$, $p < 0.0001$.)

number of eggs surviving to the third instar. These comparisons are valid if it can be assumed that the bias associated with individuals of unknown age is equal for all comparison groups. This was tested by comparing the latency to hatch, which is a measure of age distribution, among treatment groups. Latency to hatch did not differ among treatments (ANOVA: $F = 0.14$, $df = 3, 541$, $p = 0.9368$).

Annual survivorship of monarch larvae varied only slightly (Figure 17). Among control eggs, survivorship varied from 13.9% in 2017 to 10% in 2018. These differences were not statistically significant (2x3 Contingency Table, Chi-Square = 1.4806, $df = 2$, $p = 0.477$).

Monarch survival to the third instar was compared among treatments for 2017 and 2018 (Figure 18). In 2017, survival was between 12.2% and 15.5% for all treatments except the RIFA enhanced treatment (Figure 18) where survival was 6.6%. This difference was not statistically significant (4x2 Contingency Table, $X2 = 3.46$, $df = 3$, $p = 0.326$). In 2017, there was no difference among control and RIFA suppressed treatments and the highest survivorship was in the control treatment with traps (Figure 18). In 2018, survivorship in the RIFA suppressed treatment was 16.2%, 10% among controls, and 5.4% in the RIFA enhanced treatment. These differences were statistically significant (4x2 Contingency Table, $X2 = 17.53$, $df = 3$, $p = 0.0005$). However, when the RIFA enhanced treatment was excluded there were no statistically significant differences among the control treatments and the RIFA suppressed treatments (3x2 Contingency Table, $X2 = 2.42$, $df = 2$, $p = 0.2986$). Similar trends occurred when both years were combined (Figure 19). There were significant differences among treatments (4x2 Contingency Table, $X2 = 9.67$, $df = 3$, $p = 0.0215$). However, when the RIFA enhanced treatment was removed from the analysis, there were no statistically significant differences between the control treatments and the RIFA suppressed treatment (3x2 Contingency Table, $X2 = 1.39$, $df = 2$, $p = 0.4988$). The inference of these analyses is that the effect of suppressing of RIFA on monarch survival may vary between years. In some years RIFA suppression will have no effect on survival, in other years RIFA suppression results in a slight increase in monarch survival. It is important to note that this latter effect occurred in 2018 when the overall abundance of RIFA was lowest. Monarch survival is inhibited when RIFA are induced to occupy the host plant.

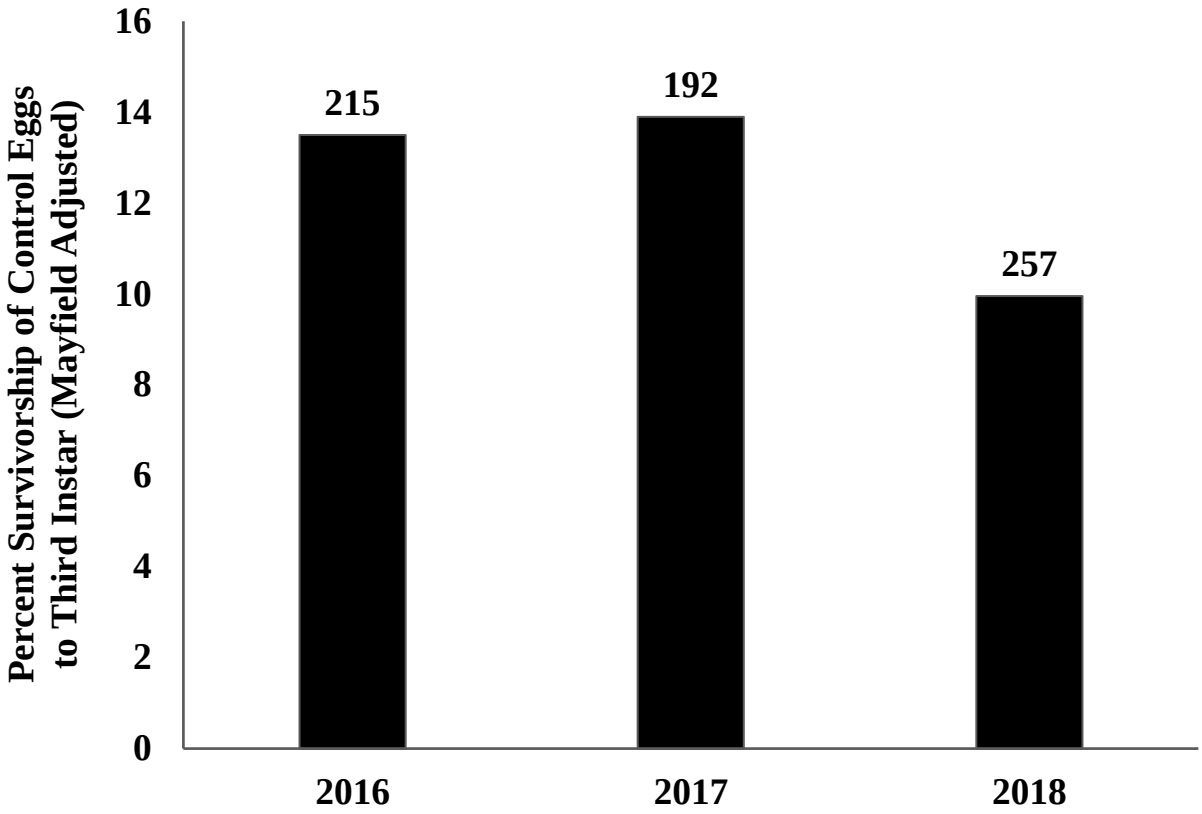

Figure 17. Percent survivorship of monarch eggs to the third instar. These data are corrected for bias resulting from using individuals of unknown eggs by using the Mayfield method (see text). Numbers over bars indicate sample sizes.

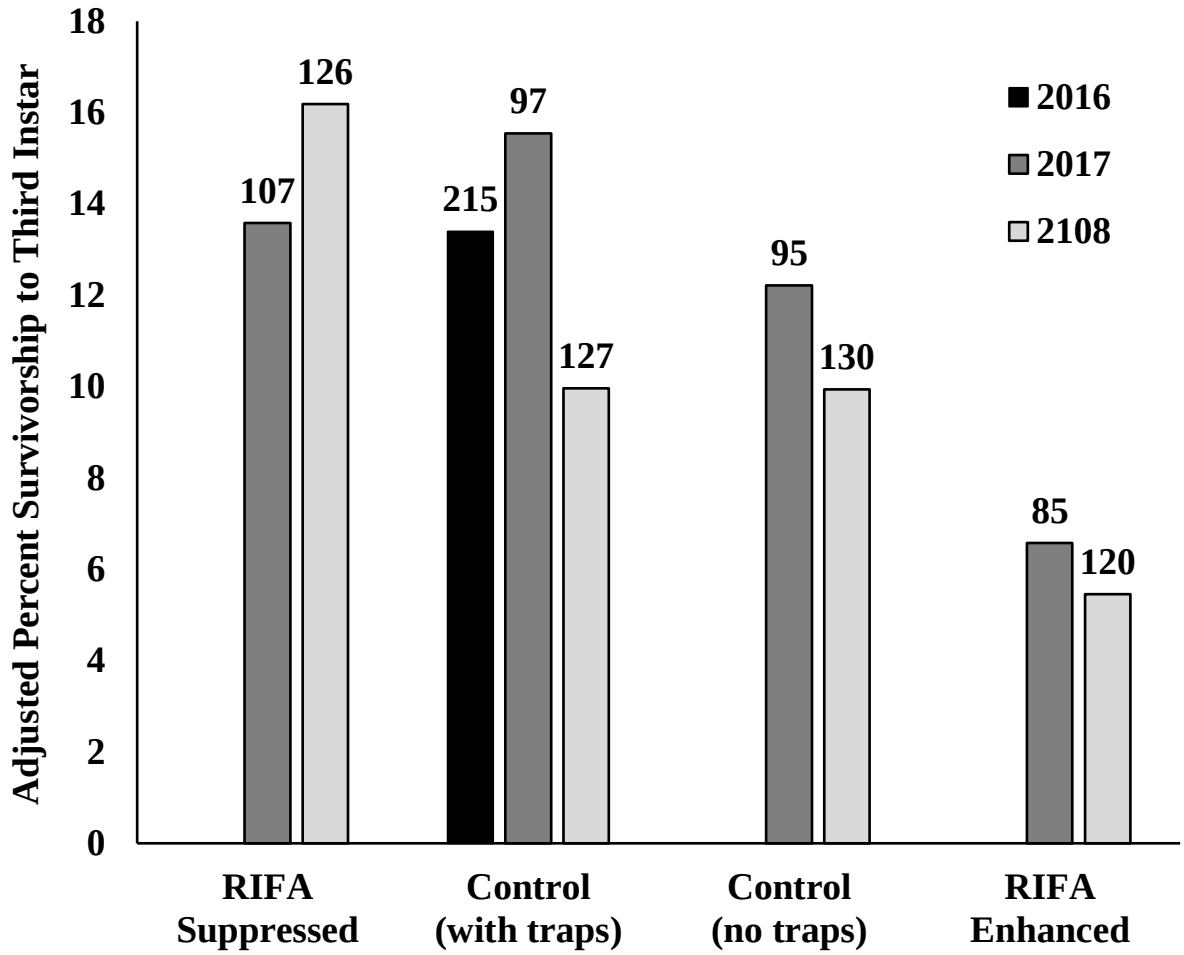

Figure 18. Effect of RIFA treatments on monarch egg and larval survival to the third instar separated by treatment and year. Survivorship is expressed as Mayfield adjusted estimates based on individuals initially found as eggs. Numbers over bars indicate the number of eggs used to calculate survivorship for each year and treatment.

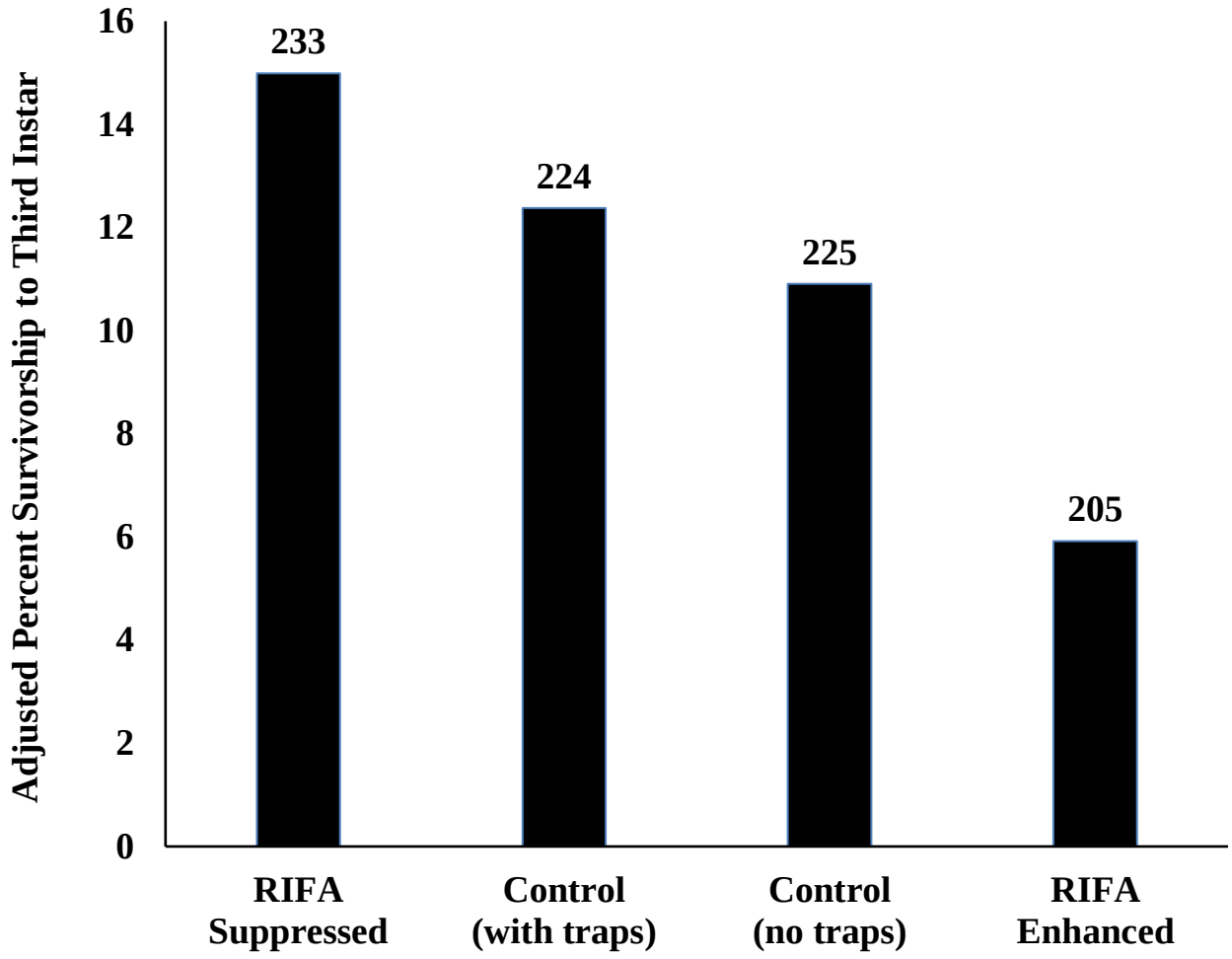

Figure 19. Effect of RIFA treatments on monarch egg and larval survival to the third instar combined for both years of the study. Survivorship is expressed as Mayfield adjusted estimates based on individuals initially found as eggs. Numbers over bars indicate the number of eggs used to calculate survivorship for each year and treatment.

The RIFA abundance measures were compared for host plants upon which eggs survived to the third instar and those upon which eggs did not survive to the third instar (Table 2). For these analyses eggs from all treatments and years were combined. There was no relationship between survival and the distance of the host plant to the nearest RIFA mound, the number of RIFA mounds within 4m, or the total volume of RIFA mounds within 4m of host plant (Table 2). There was a tendency for fewer RIFA to be caught in traps adjacent to host plants upon which monarch eggs survived (Table 2). Similar results are obtained when only control plants are included in the analysis except that, for control eggs, there was no relationship between survival and the number of RIFA captured in traps adjacent to the host plants (ANOVA, $F = 1.40$, $df = 1, 221$, $P = 0.2377$). Stepwise logistic regression on the control eggs failed to find any model that explained monarch mortality based on RIFA abundance measures.

For the data that combined all treatments, there was a relationship between the number of RIFA found on a host plant and survival. Plants upon which monarch eggs did not survive had more RIFA than did plants upon which monarch eggs did survive (Table 2). However, this relationship was not linear. It was found that when the data were divided into RIFA abundance classes, the highest survivorship occurred on host plants that had low numbers of RIFA (Figure 20). This trend occurred when all treatments were combined (Figure 20A) as well as when only the control data were included (Figure 20B). Consequently, low numbers of RIFA on host plants favored increased survival of monarch eggs. Host plants with no RIFA and host plants upon which RIFA were more common had lower egg survivorship.

c. Host Plant Arthropod Community

Some eggs were infected with parasitic wasps (Hymenoptera, Apocrita, Trichogramma) and failed to hatch. In 2017, 20 of 384 eggs were parasitized (5.2%) and in 2018, 11 of 504 eggs were parasitized (2.2%). For analyses of the influence of the plant arthropod community on monarch survival,

Table 2. Comparisons of RIFA abundance measures on and adjacent to host plants upon which eggs survived to the third instar (Lived) and host plants where the eggs died prior to the third instar (Died). This analysis includes all eggs from all treatment groups in all years.

	Mean Lived ± SE (n=112)	Mean Died ± SE (n=744)	ANOVA F (df = 1, 854)	P
Distance of host plant to nearest RIFA mound (cm)	671.42 ± 90.42 (n = 141)	562.37 ± 32.34 (n = 928)	1.46	0.2267
Number of RIFA mounds within 4.0 m of host plant	2.05 ± 0.19	2.04 ± 0.06	0.0	0.9730
Volume of RIFA mounds (cm^3) within 4.0 m of host plant	19154 ± 3479	18474 ± 1218	0.04	0.8422
Number of RIFA captured in traps adjacent to host plant	0.73 ± 0.14 (n = 84)	1.37 ± 0.12 (n = 552)	3.86 (df = 1, 634)	0.0499
Number of RIFA observed on host plant	0.61 ± 0.16	1.38 ± 0.10	7.92	0.0050

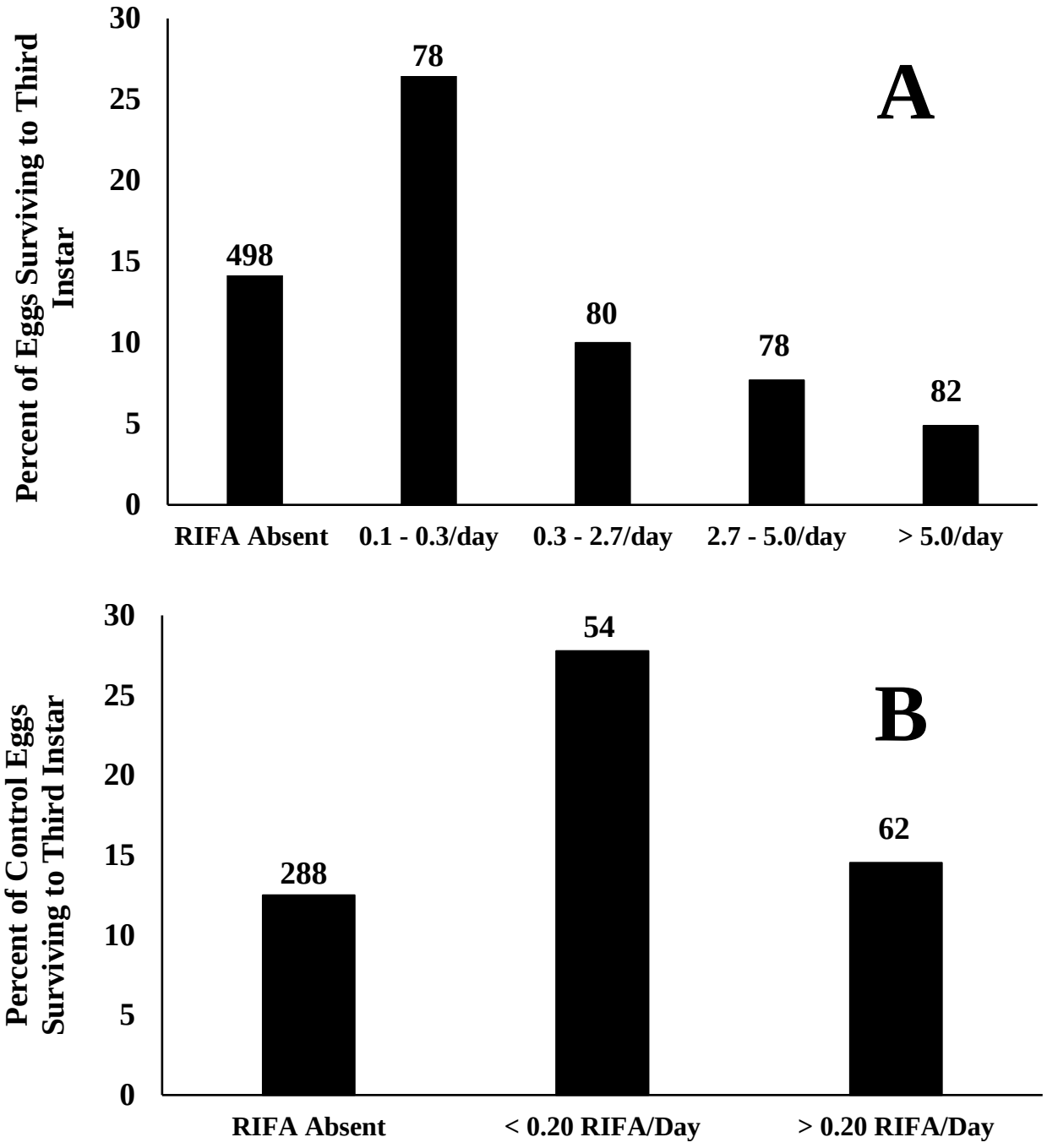

Figure 20. Survival of eggs to the third instar based on RIFA abundance class. A low abundance of RIFA was associated with the greatest chance that monarch eggs would reach the third instar. Numbers over bars are sample sizes. A. Eggs on plants in all treatment groups (2x5 Chi-square Contingency Table; df =4, P = 0.0004). B. Eggs on control plants (2x3 Chi-square Contingency Table; df =2, P = 0.015).

parasitized eggs were eliminated. Five individuals on plants browsed by rabbits in 2017 and 19 individuals on plants browsed by rabbits in 2018 were also eliminated. In 2018, 18 eggs on plants that died due to trampling or wind damage were eliminated. Analyses of plant arthropods were thus initially based on 816 eggs on 529 plants; 359 eggs on 250 plants in 2017, and 457 eggs on 279 plants in 2018.

During the course of daily host plant monitoring, 48,827 individuals, representing 86 different types of arthropods, were recorded, revealing a rather rich community (Appendix 1). There were 28 different types of predatory arthropods, and four of the five most abundant arthropods found on host plants were predators. There were eight types of milkweed herbivores (ten including monarchs and leaf miners). The remaining 50 taxa were either feeding on nectar or were transient species (Appendix 1).

Shannon Entropy Indices (Lin 1991) were used to calculate the effective number of taxa (Leinster and Cobbold 2012, Chao et al. 2014) and evenness (Jost 2010) for each treatment (Figure 20). RIFA may have cascading effects on community structure because suppression of RIFA resulted in a higher number of effective taxa and higher evenness. However, drawing RIFA onto the plants did not have much effect on either the effective number of taxa or evenness (Figure 21).

It is evident from the raw counts that not all arthropods occurred on all plants and even abundant groups did not occur on all plants (Appendix 1). Though aphids were the most abundant arthropod, jumping spiders were the most frequent arthropod (Appendix 1). Jumping spiders were associated with just under half of all monarch eggs or larvae (Relative frequency = 47.5%), whereas aphids were associated with less than a third of all monarch eggs or larvae (Relative frequency = 31.0%). Sixty-seven of the 86 taxa (78%) had frequencies of less than 10% (Appendix 1). To avoid sparse data bias (Greenland et al. 2016), some groups had to be combined. In doing so, an effort was made to combine ecologically similar taxa. By combining the arthropods this way, the 86 taxa were reduced to 15 groups (Appendix 2). Despite this, the data were still extremely sparse (Greenland et al. 2016) because many arthropods, particularly predatory arthropods other than ants, frequently occurred as single individuals associated with less than half of the plants. This type of data causes grossly erroneous estimates of effect sizes due to sparse data bias (Greenland et al. 2016).

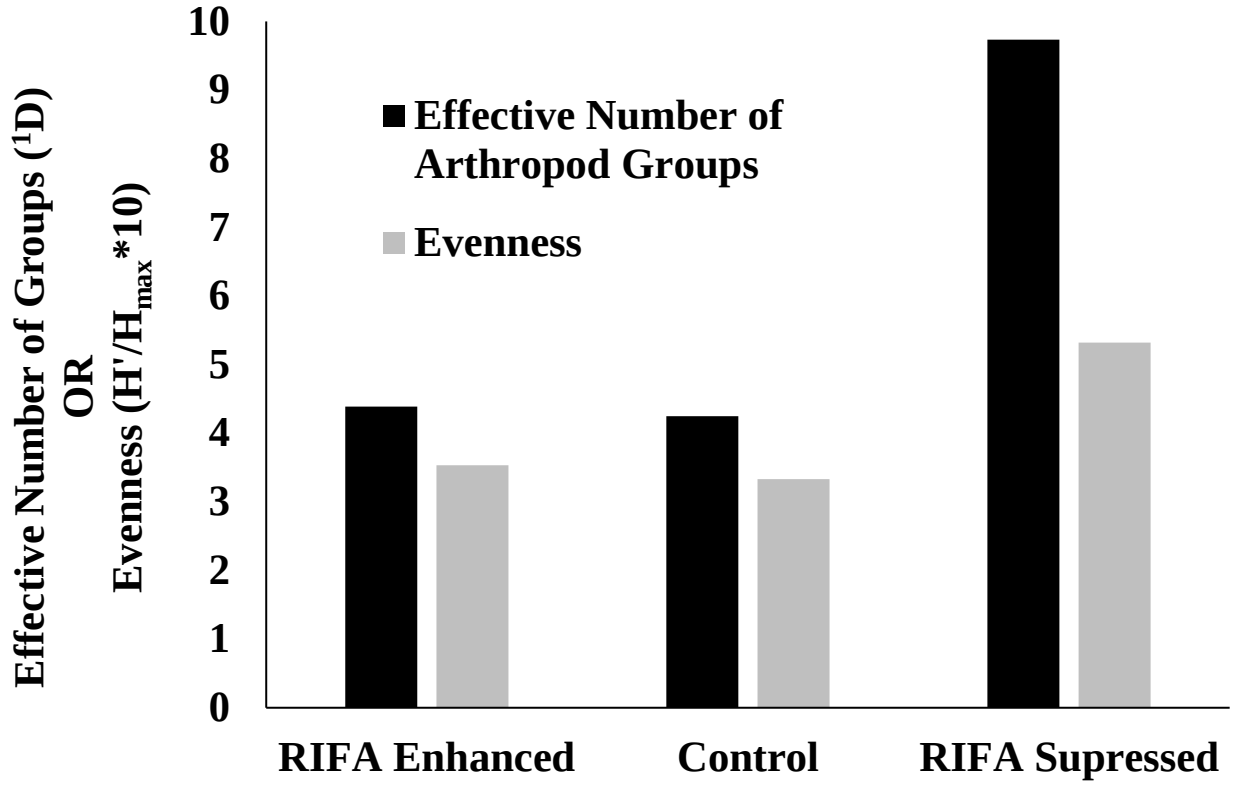

Figure 21. Effective number of arthropod groups and evenness of arthropod communities on host plants relative to treatment. Effective number of arthropod groups and community evenness were calculated using the Shannon Entropy Index.

To further reduce sparse data bias, for many analyses, plants where no arthropods were observed were eliminated because they did not contribute to understanding how particular arthropods interacted in the community. In addition, to accommodate sparse data and unbalanced samples sizes, Kruskal-Wallis nonparametric ANOVAs were used to compare means. Lastly, since arthropod counts were based on daily records, the number of arthropods recorded on a plant was divided by the number of days the plant was observed to mitigate biases resulting from differences in how long individual plants were observed.

The relationship between arthropod abundance on host plants and monarch survival was evaluated for control plants that had at least one arthropod present (Table 3). Monarch larvae that survived to the third instar occupied plants that, on average, had fewer Dermestid and Chrysomelid beetles and a greater abundance of small Diptera. These host plants also tended to hold more other arthropods, more small beetles, and more herbivores in general. This indicates that prey populations on host plants play a larger, albeit variable, role in monarch survival than do predator populations.

The pair-wise comparisons in Table 3 do not account for potential interactions among arthropod groups (indirect effects or partial correlations), nor do they identify which combination of arthropod groups predict monarch survival. Stepwise logistic regression was run using Corrected Akaike's Information Criteria (AICc) to determine which combination of arthropod groups best predicted monarch survival on control plants. Three models fit the selection criteria (Table 4). The best of these models (Table 5) included three arthropod groups. Other ants and all other arthropods had positive effects on monarch survival, whereas all other predators not including ants and jumping spiders had a negative effect on monarch survival (Table 5). This model differs from the pair-wise analysis in that it shows that some predators, particularly other predators not including ants and jumping spiders, can reduce monarch survival. Importantly, RIFA do not figure into this model. However, the model is weak as the concordance is only 55%. Furthermore, the only parameter that is statistically significant is the group all other arthropods, a group composed entirely of non-predatory arthropods (see Appendix 2). To this extent, the logistic regression model is consistent with the pair-wise comparisons of Table 3.

Table 3. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control host plants that held at least one arthropod. Comparisons based on Kruskal-Wallis ANOVA. Significant differences highlighted in yellow. Near significant differences (at $\alpha = 0.05$) highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 55)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 304)	X^2 (df =1)	P-value
Hemiptera, Aphidoidea	3.1632 ± 1.9465	5.7262 ± 2.2037	0.0697	0.7918
Formicidae, *Monomorium minimum*	0.2839 ± 0.1164	0.2513 ± 0.0606	0.0001	0.9942
Formicidae, *Solenopsis invicta*	0.3801 ± 0.1393	0.7060 ± 0.1522	1.9267	0.1651
Formicidae, Other ants	1.1405 ± 1.0973	0.1000 ± 0.0402	0.0583	0.8092
Coleoptera, Curculionidae	0.2214 ± 0.0744	0.1582 ± 0.0266	0.8472	0.3573
All Other Arthropods	0.1358 ± 0.0345	0.0878 ± 0.0099	3.2046	0.0734
Other Predators Not Including Ants and Jumping Spiders	0.0902 ± 0.0138	0.1202 ± 0.0093	0.1856	0.6666
Araneae, Salticidae	0.1012 ± 0.0171	0.1220 ± 0.0125	0.1926	0.6607
Arachnida, Acari	0.2796 ± 0.2283	0.2299 ± 0.1220	0.3913	0.5316
Diptera < 5 mm	0.0727 ± 0.0160	0.0604 ± 0.0092	4.4884	0.0341
Lygaeidae, *Oncopeltus fasciatus*	0.0696 ± 0.0244	0.0447 ± 0.0080	1.9983	0.1575

Table 3 Continued. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control host plants that held at least one arthropod. Comparisons based on Kruskal-Wallis ANOVA. Significant differences highlighted in yellow. Near significant differences (at $\alpha = 0.05$) highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 55)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 304)	X^2 (df =1)	P-value
Coleoptera, Chrysomelidae	0.0809 ± 0.0157	0.0853 ± 0.0112	4.3469	0.0371
Hemiptera, Auchenorrhyncha	0.0480 ± 0.0109	0.0687 ± 0.0086	0.6235	0.4298
Coleoptera, Dermestidae	0.0272 ± 0.0085	0.0336 ± 0.0109	11.4669	0.0007
Other Milkweed Herbivores	0.0199 ± 0.0063	0.0207 ± 0.0038	1.2155	0.2702
Coleoptera Unidentified	0.0195 ± 0.0069	0.0168 ± 0.0083	3.3811	0.0659
All Herbivores	1.0150 ± 0.2432	0.8373 ± 0.1293	3.6975	0.0545
All Predators	1.9958 ± 1.1408	1.2995 ± 0.1868	0.0136	0.9072
All Arthropods	3.0109 ± 1.1701	2.1368 ± 0.2445	0.6119	0.4341

Table 4. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on control host plants that held at least one arthropod. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
Other Ants, All Other Arthropods, All Predators Except Ants and Jumping Spiders	306.847	0	0.483	8.7890	0.0322
Other Ants, All Other Arthropods	307.843	0.996	0.294	5.7361	0.0568
Other Ants	308.395	1.548	0.223	3.1395	0.0764

Table 5. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups found on control host plants that held at least one arthropod. Concordance of this model was 55%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.6802	0.1904	77.8824	<.0001
Other Ants	1	0.0692	0.0620	1.2439	0.2647
All Other Arthropods	1	1.3725	0.6665	4.2411	0.0395
All Predators Except Ants and Jumping Spiders	1	-1.9137	1.1822	2.6203	0.1055

The finding that non-predatory arthropods affect monarch survivorship suggests that indirect density-dependent effects might be occurring. One way to evaluate density dependent effects is examine the relationship between mortality and predator pressure, measured as the proportion of arthropods on a plant that are predators. For this analysis, only host plants that held at least one arthropod were included and host plants that did not have any predators were also eliminated from the data. There was a negative relationship between the proportion of arthropods on a plant that were predators and the total number of non-predatory arthropods on the plant (Figure 22). When the number of non-predatory arthropods on the plant was high, the proportion of predators on the plant was low. Therefore, predator pressure was also low when non-predatory arthropod populations were high. The survival of monarch eggs was higher on host plants that exhibited low predator pressure than it was on host plants with high predator pressure (Figure 23). Interestingly, monarch eggs and larvae on host plants with low predator pressure also had higher survivorship than did monarch eggs and larvae on host plants with no predator pressure.

The data were divided into host plants for which predator pressure was low and host plants for which predator pressure was high. Stepwise logistic regression was used to determine which arthropods best predicted monarch egg survival at each level of predator pressure. When predator pressure was low, the procedure identified three models that met the criteria for acceptance (Table 6). All of these models included RIFA, and the best fit model included only RIFA which had a negative impact on monarch survival (Table 7). However, it is important to point out that the overall model is, at best marginally significant ($p = 0.0513$), the parameter estimate for RIFA is not significant ($p = 0.1363$) and the model's concordance is only 31.6%. Furthermore, owing to sparse data, the confidence interval for this parameter estimate of RIFA approached infinity indicating that the model likely over-estimates the effects of RIFA on monarch survival. The conclusion is that, when predator pressure is low (i.e. fewer predators per arthropod on the plant), RIFA may negatively impact monarch egg and larval survival, however, this effect is, at best, very weak.

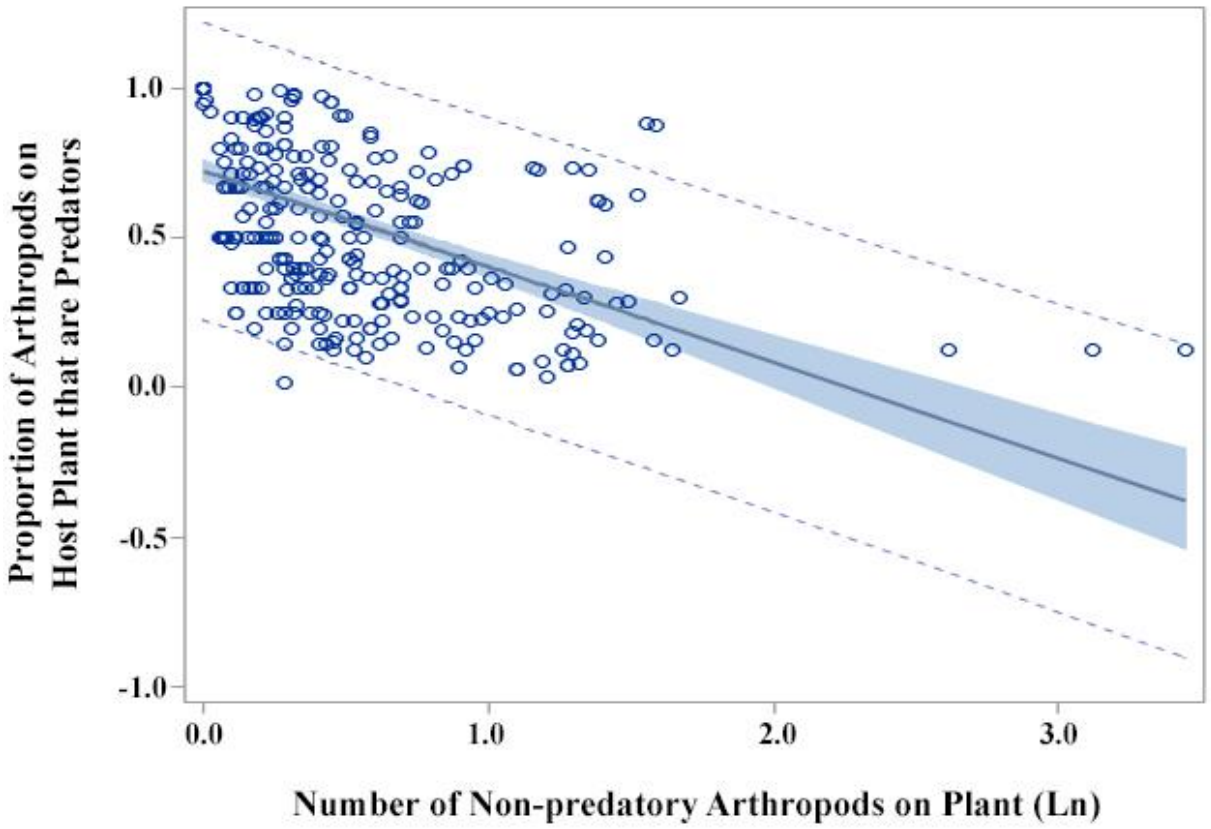

Figure 22. Relationship between the proportion of predators on a control host plant and the number of non-predatory arthropods on the same host plant. Natural log was used to transform the numbers of non-predatory arthropods on the plant. Shaded area represents the 95% confidence interval, dotted lines represent the 95% prediction limits. Formula for fitted line: $Y = -0.319X + 0.723$. Linear Regression Statistics: $F = 120.26$, $df = 1, 312$, $p = < 0.0001$, $r^2 = 0.28$).

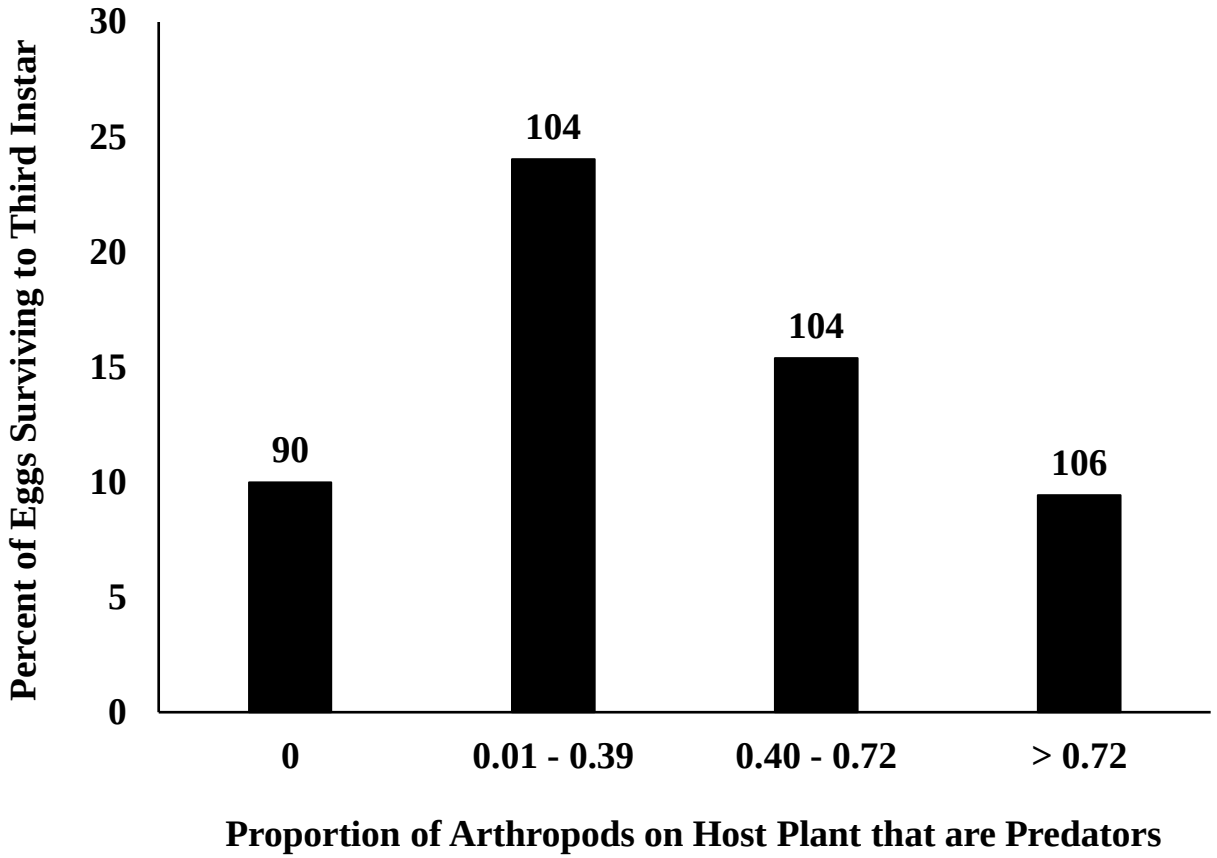

Figure 23. Control host plants upon which monarch larvae survived to the third instar had fewer predators relative to the total number of arthropods on the plant than did host plants upon which monarch larvae failed to reach the third instar. Numbers over bars are sample sizes. Chi-square, 2x4 Contingency Table, $X^2 = 11.0181$, df = 3, p = 0.0116.

Table 6. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on control host plants. Analysis restricted to plants where the proportion of predators relative to all arthropods on the plants was low (< 0.548). A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
RIFA	153.523	153.52	0.379	3.7998	0.0513
RIFA, All Predators except ants and jumping spiders	153.884	153.88	0.316	5.5449	0.0625
RIFA, All Predators except ants and jumping spiders, All other Arthropods	153.951	153.95	0.306	7.6117	0.0548

Table 7. Summary of the best fit model based on AICc using logistic regression of survival of monarch eggs or larvae based on arthropod groups found on control host plants where the proportion of predators to non-predators was low. Concordance of this model was 31.6%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.2480	0.2236	31.1489	<0.0001
RIFA[a]	1	-3.6684	2.4628	2.2188	0.1363

[a]Parameter confidence interval approaches infinity

When predator pressure is high (i.e. more predators per non-predatory arthropod on the plant), five models met the selection criteria (Table 8). The best model, included unknown beetles, other ants, weevils, and all other predators except ants and jumping spiders (Table 9). Of these, only predators other than ants and jumping spiders had a negative impact on monarch survival. However, this parameter estimate had a confidence interval approaching infinity, indicating that it is overestimated. Similarly, unknown beetles had a strong positive impact on monarch survival, but this is also overestimated. Other ants had a positive effect on monarch survival, but this effect was extremely weak and not statistically significant. The conclusion from the model is that more beetles and, in particular, more weevils lead to higher survival of monarch larvae when predator pressure is high. This result supports the idea that when predator pressure is high, increased numbers of alternate prey favor higher survivorship for monarch eggs and larvae. Though other predators may influence monarch survival, RIFA do not have an impact on monarch survival when predator pressure is high.

Because biotic and abiotic factors varied among years (see section *a. Phenological and Meteorological Considerations*) analyses were conducted to determine whether the arthropods associated with monarch mortality also varied among years. There were more arthropods in 2017 than in 2018 (Table 10). The exceptions to this rule were aphids which were much more abundant in 2018 and RIFA which, to a lesser extent, were also more abundant on the host plants in 2018. However, the apparent greater abundance of RIFA in 2018 is due to three eggs on a single plant that was heavily infested with aphids and, consequently, had large numbers of RIFA on it. If these three eggs are removed from the analysis, then there are significantly fewer RIFA on plants in 2018 than was observed in 2017 (Kruskal-Wallis ANOVA, Chi-square Approximation, $X^2 = 28.3991$, df = 1, $P < 0.0001$).

Differences between years makes it likely that the arthropods most affecting monarch survival also vary between years. The data were therefore analyzed separately for each year. Pairwise comparisons of arthropods on host plants upon which larvae survived and on host plants where larvae died showed no evidence of relationships between predatory arthropods and monarch survival for either year (Table 11, Table 12). In 2017, host plants that had larvae survive to the third instar had greater

Table 8. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on host plants. Analysis restricted to plants where the proportion of predators relative to all arthropods on the plants was high (> 0.548). A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
Unknown Beetles, Other Ants, Curculionidae, All Other Predators Except Ants and Jumping Spiders	117.758	0	0.445	16.5436	0.0024
Unknown Beetles, Other Ants, Curculionidae, All Other Predators Except Ants and Jumping Spiders, Little Black Ants	118.146	0.388	0.366	18.3760	0.0025
Unknown Beetles, Other Ants, Curculionidae	120.755	2.998	0.099	11.1910	0.0107
Unknown Beetles, Other Ants	121.700	3.942	0.062	8.1121	0.0173
Unknown Beetle	123.312	5.554	0.028	4.3941	0.0361

Table 9. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups found on host plants where the proportion of predators to non-predators was high. Concordance of this model was 68.9%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.8627	0.3294	31.9771	<.0001
Other Ants	1	0.0722	0.0602	1.4366	0.2307
Curculionidae	1	0.7612	0.3530	4.6515	0.0310
All Other Predators Except Ants and Jumping Spiders[a]	1	-3.9177	2.0419	3.6811	0.0550
Unknown Beetles[a]	1	18.0545	6.3723	8.0274	0.0046

[a]Parameter confidence interval approaches infinity

Table 10. Comparison of the abundance of arthropod groups found on control plants among years. Statistical comparisons based on Kruskal-Wallis ANOVA. Arthropods that were more abundant in 2018 are highlighted in yellow. Arthropods that were more abundant in 2017 are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Mean Number on Control plants in 2017 (Mean ± SE, N = 169)	Mean Number on Control plants in 2018 (Mean ± SE, N = 190)	X^2 (df =1)	P-value
Hemiptera, Aphidoidea	0.0274 ± 0.0083	10.0531 ± 3.5395	72.6714	<0.0001
Formicidae, *Monomorium minimum*	0.8252 ± 0.2280	0.5056 ± 0.1407	25.6749	<0.0001
Formicidae, *Solenopsis invicta*	0.2518 ± 0.0560	0.2603 ± 0.0898	24.2411	<0.0001
Formicidae, Other ants	0.0450 ± 0.0148	0.4501 ± 0.3233	0.7053	0.4010
Coleoptera, Curculionidae	0.2486 ± 0.0458	0.0961 ± 0.0237	25.3127	<0.0001
All Other Arthropods	0.1295 ± 0.0173	0.0646 ± 0.0101	10.1379	0.0015
Other Predators Not Including Ants and Jumping Spiders	0.1434 ± 0.0126	0.0909 ± 0.0104	16.6907	<0.0001
Araneae, Salticidae	0.1531 ± 0.0187	0.0883 ± 0.0117	6.2830	0.0122
Arachnida, Acari	0.0729 ± 0.0132	0.3839 ± 0.2052	2.7649	0.0964
Diptera < 5 mm	0.1017 ± 0.0163	0.0272 ± 0.0036	12.3857	0.0004
Lygaeidae, *Oncopeltus fasciatus*	0.0796 ± 0.0147	0.0209 ± 0.0060	16.7245	<0.0001

Table 10 Continued. Comparison of the abundance of arthropod groups found on control plants among years. Statistical comparisons based on Kruskal-Wallis ANOVA. Arthropods that were more abundant in 2018 are highlighted in yellow. Arthropods that were more abundant in 2017 are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Mean Number on Control plants in 2017 (Mean ± SE, N = 169)	Mean Number on Control plants in 2018 (Mean ± SE, N = 190)	X^2 (df =1)	P-value
Coleoptera, Chrysomelidae	0.1355 ± 0.0160	0.0394 ± 0.0107	53.5761	<0.0001
Hemiptera, Auchenorrhyncha	0.0807 ± 0.0131	0.0521 ± 0.0080	2.8425	0.0918
Coleoptera, Dermestidae	0.0604 ± 0.0193	0.0079 ± 0.0031	4.5867	0.0322
Other Milkweed Herbivores	0.0314 ± 0.0065	0.0109 ± 0.0024	3.3011	0.0692
Coleoptera Unidentified	0.0365 ± 0.0149	0.0000 ± 0.0000	36.6087	<0.0001
All Herbivores	1.0150 ± 0.0773	0.7306 ± 0.2072	58.8340	<0.0001
All Predators	1.4185 ± 0.2688	1.3952 ± 0.3750	32.8612	<0.0001
All Arthropods	2.4335 ± 0.3079	2.1258 ± 0.4386	44.1815	<0.0001

Table 11. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control host plants in 2017. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. Near significant differences (at $\alpha = 0.05$) are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 28)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 141)	X^2 (df =1)	P-value
Hemiptera, Aphidoidea	0.0032 ± 0.0032	0.0322 ± 0.0098	1.6280	0.2020
Formicidae, *Monomorium minimum*	0.6186 ± 0.2588	0.8663 ± 0.2686	0.0336	0.8545
Formicidae, *Solenopsis invicta*	0.3678 ± 0.2041	0.2288 ± 0.0538	0.5666	0.4516
Formicidae, Other ants	0.0524 ± 0.0256	0.0435 ± 0.0170	1.3498	0.2453
Coleoptera, Curculionidae	0.3777 ± 0.1373	0.2230 ± 0.0476	1.9837	0.1590
All Other Arthropods	0.1798 ± 0.0606	0.1196 ± 0.0169	0.7875	0.3749
Other Predators Not Including Ants and Jumping Spiders	0.1306 ± 0.0222	0.1460 ± 0.0145	0.0341	0.8534
Araneae, Salticidae	0.1511 ± 0.0292	0.1535 ± 0.0217	1.3460	0.2460
Arachnida, Acari	0.0613 ± 0.0227	0.0752 ± 0.0152	0.2737	0.6008
Diptera < 5 mm	0.1223 ± 0.0281	0.0976 ± 0.0188	5.2268	0.0222
Lygaeidae, *Oncopeltus fasciatus*	0.0987 ± 0.0434	0.0758 ± 0.0154	0.6509	0.4198

Table 11 Continued. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control host plants in 2017. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. Near significant differences (at $\alpha = 0.05$) are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 28)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 141)	X^2 (df =2)	P-value
Coleoptera, Chrysomelidae	0.1329 ± 0.0217	0.1361 ± 0.0187	3.3112	0.0688
Hemiptera, Auchenorrhyncha	0.0566 ± 0.0195	0.0855 ± 0.0152	0.0254	0.8734
Coleoptera, Dermestidae	0.0347 ± 0.0143	0.0655 ± 0.0229	3.9254	0.0476
Other Milkweed Herbivores	0.0198 ± 0.0102	0.0337 ± 0.0075	0.2619	0.6088
Coleoptera Unidentified	0.0383 ± 0.0127	0.0361 ± 0.0177	2.9385	0.0865
All Herbivores	1.1581 ± 0.1838	0.9866 ± 0.0852	1.8892	0.1693
All Predators	1.3204 ± 0.3427	1.4380 ± 0.3153	0.1433	0.7050
All Arthropods	2.4785 ± 0.4884	2.4246 ± 0.3566	0.6834	0.4084

Table 12. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control plants in 2018. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. Near significant differences (at $\alpha = 0.05$) are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 27)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 163)	X^2 (df =1)	P-value
Hemiptera, Aphidoidea	6.4401 ± 3.9012	10.6516 ± 4.0763	1.6686	0.1964
Formicidae, *Monomorium minimum*	0.1327 ± 0.0730	0.5673 ± 0.1632	0.0464	0.8295
Formicidae, *Solenopsis invicta*	0.1969 ± 0.1088	0.2707 ± 0.1032	0.6589	0.4170
Formicidae, Other ants	2.2689 ± 2.2355	0.1488 ± 0.0734	0.7952	0.3725
Coleoptera, Curculionidae	0.0593 ± 0.0338	0.1022 ± 0.0271	0.2933	0.5881
All Other Arthropods	0.0903 ± 0.0304	0.0604 ± 0.0107	2.7470	0.0974
Other Predators Not Including Ants and Jumping Spiders	0.0482 ± 0.0122	0.0980 ± 0.0119	1.2278	0.2678
Araneae, Salticidae	0.0495 ± 0.0109	0.0948 ± 0.0135	0.7127	0.3986
Arachnida, Acari	0.5059 ± 0.4649	0.3637 ± 0.2269	0.0429	0.8360
Diptera < 5 mm	0.0214 ± 0.0064	0.0282 ± 0.0041	0.0058	0.9394
Lygaeidae, *Oncopeltus fasciatus*	0.0395 ± 0.0206	0.0178 ± 0.0062	1.1950	0.2743

Table 12 Continued. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on control plants in 2018. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. Near significant differences (at $\alpha = 0.05$) are highlighted in green. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 27)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 163)	X^2 (df =1)	P-value
Coleoptera, Chrysomelidae	0.0270 ± 0.0177	0.0414 ± 0.0122	0.0014	0.9702
Hemiptera, Auchenorrhyncha	0.0392 ± 0.0094	0.0542 ± 0.0092	1.6059	0.2051
Coleoptera, Dermestidae	0.0194 ± 0.0092	0.0059 ± 0.0033	7.9433	0.0048
Other Milkweed Herbivores	0.0200 ± 0.0077	0.0094 ± 0.0025	5.3775	0.0204
Coleoptera Unidentified	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000	1.0000
All Herbivores	0.8667 ± 0.4607	0.7081 ± 0.2296	1.4956	0.2214
All Predators	2.6963 ± 2.3113	1.1796 ± 0.2171	0.8377	0.3601
All Arthropods	3.5630 ± 2.3480	1.8877 ± 0.3355	0.0008	0.9774

numbers of small Diptera and unknown beetles and had lower numbers of Chrysomelid and Dermestid beetles (Table 11). In 2018, host plants upon which larvae survived to the third instar had greater numbers of other arthropods, Dermestid beetles, and other milkweed herbivores. All of these differences involved non-predatory arthropod groups.

Stepwise logistic regressions were performed to identify the combinations of arthropod groups on control plants that best predicted monarch survival to the third instar for each year. For the 2017 three models fit the selection criteria, but none of the model probabilities were statistically significant indicating that these models had low predictive value (Table 13). The best model included only aphids and weevils (Table 14). In this analysis, host plants with more weevils and fewer aphids were more likely to have monarchs survive to the third instar. The confidence interval for the parameter estimate associated with aphids approached infinity indicating that this effect is overestimated in the model. The model itself only has a concordance of 51.1%.

For the 2018 data the logistic regression procedure identified nine models that predicted monarch survivorship. Four of the five best models were statistically significant (Table 15). The best of these models included six arthropod groups (Table 16), though the parameter estimate of only one group, all other arthropods, was statistically significant. In this model, plants with fewer Chrysomelid beetles and fewer predators other than ants and jumping spiders favored monarch survival. On the other hand, increased survival was predicted to occur with increasing numbers of other ants, other milkweed herbivores, Dermestid beetles, and all other arthropods. Overall, these effects are weak. Furthermore, because their confidence intervals approached infinity, the effect sizes for other milkweed herbivores, Dermestid beetles, all other arthropods, and Chrysomelid beetles are overestimated in this model.

In summary, the arthropod data from control plants indicate that some arthropods have an impact on the survival of monarch eggs to the third instar. In all of the models analyzed, the most important predictors of monarch survival were non-predatory arthropods. These effects varied relative to predator pressure and year. In the few instances when predatory arthropods were identified as affecting monarch survival, these arthropod groups did not include RIFA. However, predator pressure is important

Table 13. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on control host plants in 2017. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
Aphids, Curculionidae	152.467	0.000	0.355	5.5295	0.0630
Aphids, Curculionidae, All Other Arthropods	152.655	0.188	0.323	7.4653	0.0585
Aphids	152.658	0.192	0.322	3.2391	0.0719

Table 14. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups found on control host plants in 2017. Concordance of this model was 51.1%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.6483	0.2308	51.0023	<.0001
Aphids[a]	1	-10.1174	8.3197	1.4789	0.2240
Curculionidae	1	0.4868	0.3084	2.4910	0.1145

[a]Parameter confidence interval approaches infinity

Table 15. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on control host plants in 2018. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
Other Ants, Other Milkweed Herbivores, Dermestids, All Predators Except Ants and Jumping Spiders, All Other Arthropods, Chrysomelidae,	155.277	0.000	0.230	14.8508	0.0214
Other Ants, Other Milkweed Herbivores, Dermestids, All Predators Except Ants and Jumping Spiders, All Other Arthropods, Chrysomelidae, Jumping Spiders	155.399	0.122	0.217	16.9328	0.0178
Other Ants, Other Milkweed Herbivores, Dermestids, All Predators Except Ants and Jumping Spiders	155.470	0.193	0.209	10.3207	0.0354
Other Ants, Other Milkweed Herbivores, Dermestids, All Predators Except Ants and Jumping Spiders, All Other Arthropods, Chrysomelidae, Jumping Spiders, Large Milkweed Bugs	155.615	0.338	0.194	18.9458	0.0152
Other Ants, Other Milkweed Herbivores	156.138	0.862	0.150	5.409	0.0669

Table 16. Summary of the best fit model using logistic regression to predict survival of monarch eggs or larvae based on arthropod groups found on control host plants in 2018. Concordance of this model was 69%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.9082	0.2913	42.8984	<.0001
Other Ants	1	0.0717	0.0665	1.1637	0.2807
Other Milkweed Herbivores[a]	1	8.8502	5.1394	2.9654	0.0851
Dermestids[a]	1	4.3971	3.7309	1.3890	0.2386
All Predators Except Ants and Jumping Spiders[a]	1	-4.3109	2.4812	3.0186	0.0823
All Other Arthropods[a]	1	4.2995	2.0097	4.5769	0.0324
Chrysomelidae[a]	1	-4.0294	2.5445	2.5076	0.1133

[a]Parameter confidence interval approaches infinity

because monarch survival was higher on host plants with lower predator pressure which tends to occur when the abundance of non-predatory arthropods is high.

d. Effects of Experimental Treatments of RIFA Populations on Host Plant Arthropods

The experimental treatments appeared to affect the evenness and effective number of arthropod groups on the host plants (Figure 21). In simple pair-wise comparisons, eleven of the 16 arthropod groups showed significant variations among treatments (Table 17). Owing to the treatments themselves, RIFA were far more common on enhanced treatment host plants and least abundant on suppressed treatment host plants. However, little black ants were also reduced on host plants in the suppressed treatment. Seven of the remaining nine arthropod groups that exhibited significant differences among treatments showed either reduced abundances on host plants in the enhanced treatment or elevated abundances on host plants in the suppressed treatment group or both (Table 17). Two exceptions to this trend were small Diptera and large milkweed bugs which were more abundant on host plants in the RIFA enhanced treatment and less abundant on host plants in the RIFA suppressed treatment (Table 17). It is evident that the increased evenness and effective number of species observed on host plants in the suppressed treatment (Figure 21) is due to larger numbers of many arthropod groups and an overall greater number of non-predatory species on these plants (Table 17).

The purpose of the treatments was to determine how manipulation of RIFA densities on the host plants might affect monarch egg and larval survival. In order to make full use of the data, each host plant was treated as an individual sample unit and the host plants in all of the treatment groups were combined for further analyses. This enabled analyses of 755 eggs on host plants upon which at least one arthropod occurred. Comparisons were then made of the number of individuals in each arthropod group on host plants where monarch larvae survived to the third instar and those host plants where larvae failed to

Table 17. Arthropod abundances (mean ± standard error) on monarch host plants compared among treatments. Significant differences are highlighted in yellow. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Control Mean ± SE (n = 359)	RIFA Enhanced Mean ± SE (n = 198)	RIFA Suppressed Mean ± SE (n = 198)	Kruskal-Wallis Test X^2 (df =2)	P-value
Hemiptera, Aphidoidea	5.3335 ± 1.8895	1.1224 ± 0.7015	1.5397 ± 0.5552	0.1961	0.9066
Formicidae, *Monomorium minimum*	0.6560 ± 0.1307	0.6991 ± 0.1210	0.0190 ± 0.0063	65.6408	<0.0001
Formicidae, *Solenopsis invicta*	0.2563 ± 0.0543	4.8991 ± 0.2427	0.0019 ± 0.0009	532.5215	<0.0001
Formicidae, Other ants	0.2594 ± 0.1714	0.0980 ± 0.0362	0.0205 ± 0.0058	4.0248	0.1337
Coleoptera, Curculionidae	0.1679 ± 0.0252	0.0493 ± 0.0119	0.2206 ± 0.0426	18.6128	<0.0001
All Other Arthropods	0.0952 ± 0.0099	0.0869 ± 0.0133	0.1266 ± 0.0132	23.6608	<0.0001
Other Predators Not Including Ants and Jumping Spiders	0.1156 ± 0.0082	0.1747 ± 0.0297	0.1595 ± 0.0155	5.4681	0.0650
Araneae, Salticidae	0.1188 ± 0.0109	0.0837 ± 0.0101	0.1878 ± 0.0231	17.7620	0.0001
Arachnida, Acari	0.2375 ± 0.1089	0.0532 ± 0.0094	0.0351 ± 0.0062	3.2582	0.1961
Diptera < 5 mm	0.0623 ± 0.0081	0.1315 ± 0.0318	0.0891 ± 0.0095	22.3093	<0.0001
Lygaeidae, *Oncopeltus fasciatus*	0.0485 ± 0.0078	0.1378 ± 0.0334	0.0988 ± 0.0173	13.3554	0.0013

Table 17 Continued. Arthropod abundances (mean ± standard error) on monarch host plants compared among treatments. Significant differences are highlighted in yellow. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Control Mean ± SE (n = 179)	RIFA Enhanced Mean ± SE (n = 83)	RIFA Suppressed Mean ± SE (n = 102)	Kruskal-Wallis Test X^2 (df =2)	P-value
Coleoptera, Chrysomelidae	0.0847 ± 0.0097	0.0470 ± 0.0076	0.0941 ± 0.0166	5.8314	0.0542
Hemiptera, Auchenorrhyncha	0.0655 ± 0.0075	0.0418 ± 0.0059	0.0955 ± 0.0093	26.0688	<0.0001
Coleoptera, Dermestidae	0.0326 ± 0.0093	0.0316 ± 0.0165	0.0728 ± 0.0176	9.2158	0.0100
Other Milkweed Herbivores	0.0206 ± 0.0033	0.0339 ± 0.0080	0.0414 ± 0.0080	8.8182	0.0122
Coleoptera Unidentified	0.0172 ± 0.0071	0.0097 ± 0.0040	0.0254 ± 0.0074	6.1192	0.0469
All Herbivores	0.8645 ± 0.1156	0.6718 ± 0.0777	0.9748 ± 0.0874	23.0484	<0.0001
All Predators	1.4062 ± 0.2350	5.9545 ± 0.2680	0.3887 ± 0.0377	353.4746	<0.0001
All Arthropods	2.2707 ± 0.2734	6.6262 ± 0.2846	1.3635 ± 0.1118	284.9347	<0.0001

survive to the third instar (Table 18). In this analysis host plants that held monarch eggs or larvae that survived had fewer RIFA, Chrysomelid beetles, and leaf hoppers and more other ants, weevils, Dermestid beetles, other milkweed herbivores, and unidentified beetles. Host plants upon which monarch eggs or larvae reached the third instar held, in general, more non-predatory arthropods and fewer predatory arthropods (Table 18). This latter result is consistent with previous findings that monarch eggs and larvae were more likely to survive when predator pressure (i.e. proportion of arthropods on plant that are predators) is low (see Figure 23).

These data were further evaluated using stepwise logistic regression to determine what combination of arthropods best predicted monarch survival. This procedure found seven models that met the selection criteria and the top five, based on AICc, are shown in Table 19. All of these models include RIFA. The best model (Table 20) indicates that, for this data, RIFA had a significant negative impact on monarch survival. Similarly, little black ants also negatively impacted monarch survival, whereas other ants and weevils are predicted to positively impact monarch survival. However, the parameter estimates for these last three groups of arthropods did not reach statistical significance.

The preceding analyses indicate that RIFA are only a factor affecting monarch survival under rather limited conditions: a weak effect if predator pressure is low and, more importantly, if RIFA are experimentally drawn onto the plant. It may be instructive, therefore, to determine what attributes of the control plants are responsible for drawing RIFA onto host plants under natural circumstances. For this analysis it was presumed that RIFA are drawn onto the plant by the presence of other arthropods (as the mealworms simulated in the RIFA enhanced treatment). Because the numbers were highly skewed, the number of non-predatory arthropods on the host plant was log transformed and divided into five abundance classes based on 20% quantiles. RIFA were most abundant on host plants whose non-predatory arthropod populations fell in the top two abundance classes indicating that RIFA only occupy host plants in high numbers if there are large numbers of non-predatory arthropods present on the plant (Figure 24).

Table 18. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on the host plants. These data include all control plants and all experimental plants that had at least one arthropod. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 105)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 650)	X^2 (df =1)	P-value
Hemiptera, Aphidoidea	1.9771 ± 1.0269	3.4373 ± 1.0683	1.5416	0.2144
Formicidae, *Monomorium minimum*	0.2262 ± 0.0748	0.5445 ± 0.0809	0.0173	0.8953
Formicidae, *Solenopsis invicta*	0.6465 ± 0.1736	1.5300 ± 0.1148	5.0774	0.0242
Formicidae, Other ants	0.6216 ± 0.5748	0.0789 ± 0.0218	4.5985	0.0320
Coleoptera, Curculionidae	0.2145 ± 0.0496	0.1403 ± 0.0178	6.2873	0.0122
All Other Arthropods	0.1259 ± 0.0213	0.0973 ± 0.0071	3.1172	0.0775
Other Predators Not Including Ants and Jumping Spiders	0.1390 ± 0.0200	0.1432 ± 0.0107	0.8422	0.3588
Araneae, Salticidae	0.1127 ± 0.0144	0.1301 ± 0.0096	0.7887	0.3745
Arachnida, Acari	0.1695 ± 0.1200	0.1307 ± 0.0572	0.1591	0.6900
Diptera < 5 mm	0.0786 ± 0.0131	0.0889 ± 0.0109	2.8970	0.0887
Lygaeidae, *Oncopeltus fasciatus*	0.1175 ± 0.0304	0.0799 ± 0.0113	3.2078	0.0733

Table 18 Continued. Percent survival of monarch eggs and larva relative to the presence or absence of each arthropod group on the host plants. These data include all control plants and all experimental plants that had at least one arthropod. Statistical comparisons based on Kruskal-Wallis ANOVA. Significant differences are highlighted in dark yellow. See Appendix 2 for description of Arthropod groupings.

Arthropod Type	Number on Plants where Larvae Survived (Mean ± SE, N = 105)	Number on Plants where Larvae Did Not Survive (Mean ± SE, N = 650)	X^2 (df =1)	P-value
Coleoptera, Chrysomelidae	0.0756 ± 0.0115	0.0775 ± 0.0076	5.3286	0.0210
Hemiptera, Auchenorrhyncha	0.0661 ± 0.0093	0.0673 ± 0.0052	4.0096	0.0452
Coleoptera, Dermestidae	0.0583 ± 0.0211	0.0404 ± 0.0083	14.2642	0.0002
Other Milkweed Herbivores	0.0365 ± 0.0094	0.0284 ± 0.0036	5.1755	0.0229
Coleoptera Unidentified	0.0179 ± 0.0047	0.0173 ± 0.0046	6.2128	0.0127
All Herbivores	1.0165 ± 0.1564	0.8149 ± 0.0687	5.8903	0.0152
All Predators	1.7460 ± 0.6183	2.4268 ± 0.1492	6.6086	0.0101
All Arthropods	2.7625 ± 0.6467	3.2416 ± 0.1684	3.4626	0.0628

Table 19. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found on host plants. This data includes all experimental and control host plants that held at least one arthropod. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
RIFA, Other Ants, Curculionidae, Little Black Ants	598.369	0.000	0.281	20.6906	0.0004
RIFA, Other Ants, Curculionidae, Little Black Ants, Large Milkweed Bug, Salticidae	598.909	0.540	0.215	24.2321	0.0005
RIFA, Other Ants, Curculionidae, Little Black Ants, Large Milkweed Bug	599.044	0.675	0.201	22.054	0.0005
RIFA, Other Ants	599.513	1.144	0.159	15.4879	0.0004
RIFA, Other Ants, Curculionidae, Little Black Ants, Large Milkweed Bug, Salticidae, All Other Arthropods	599.698	1.328	0.145	25.4922	0.0006

Table 20. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups found on host plants. This data includes all experimental and control host plants that held at least one arthropod. Concordance of this model was 56%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.6666	0.1228	184.1321	<0.0001
RIFA	1	-0.1682	0.0611	7.5874	0.0059
Other Ants	1	0.0807	0.0674	1.4358	0.2308
Curculionidae	1	0.3123	0.1933	2.6103	0.1062
Little Black Ants	1	-0.1658	0.1176	1.9877	0.1586

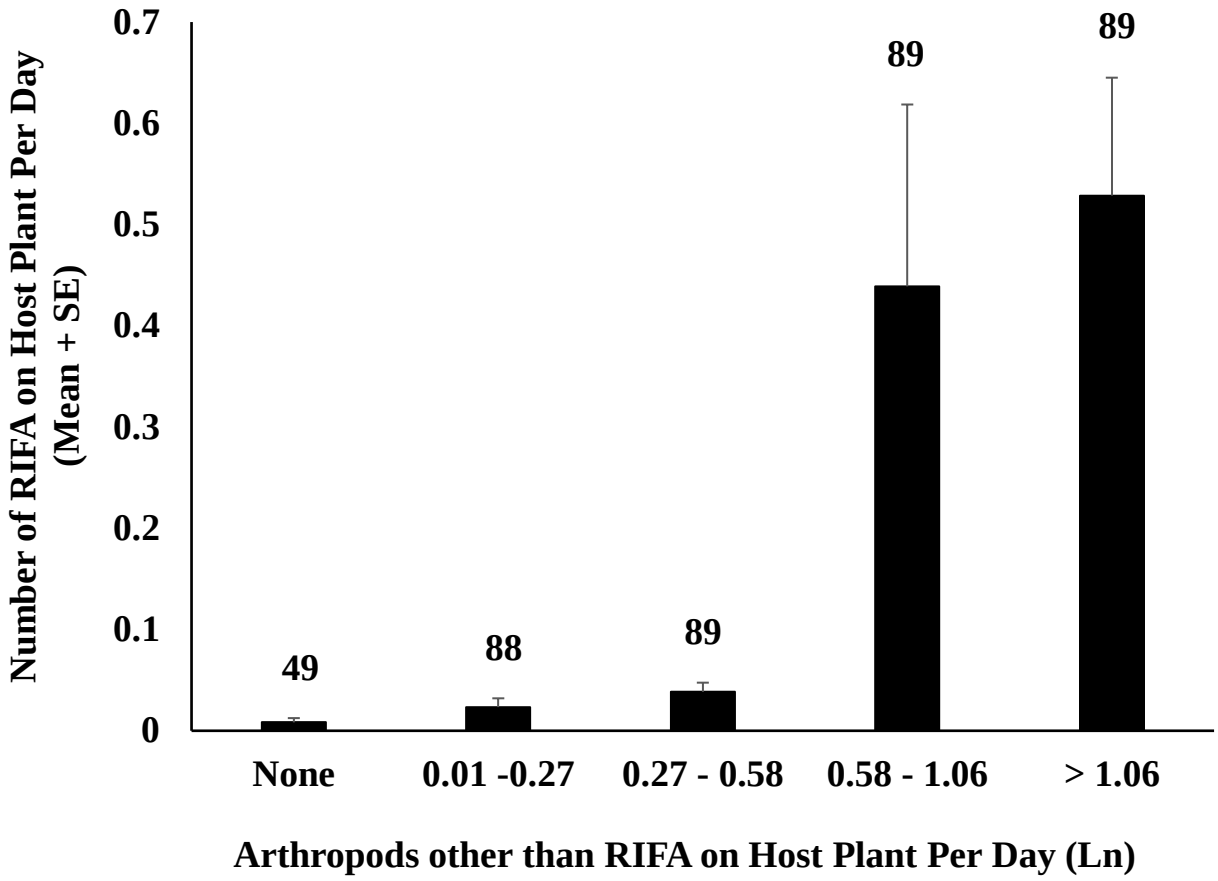

Figure 24. The number of RIFA on control plants (expressed as number of individuals observed per plant per day) based on abundance classes of all other arthropods excluding RIFA on the host plant. The data on non-predatory arthropods observed on a host plant were log-transformed and then divided into five abundance classes roughly based on 20% quantiles. Numbers above bars are sample sizes for each abundance class. ANOVA: $F = 3.63$, $df = 4, 399$, $p = 0.0064$.

Stepwise multiple regression was used to predict which non-predatory arthropod groups most strongly predicted the abundance of RIFA on control host plants that held at least one non-predatory arthropod. The five best of nine models are presented in Table 21. Four of the 10 arthropod groups used in the analyses appeared in the best (lowest AICc) model: Aphids, weevils, leaf beetles, and leafhoppers. All of these except leaf beetles had significant positive effects on RIFA numbers on the host plant (Table 22). Leaf beetles had a weak negative impact on the number of RIFA on the host plants (Table 22).

The results shown in Figure 24 and Tables 21 and 22 indicate that RIFA only ascend onto the host plant in large numbers when there are large numbers of other arthropods, particularly aphids, weevils, and leaf hoppers. It is possible that, under these circumstances, RIFA would cause of high predator pressure, a situation that would lead to high monarch mortality (see Figure 20B). To test for this, the number of RIFA on the host plants were compared among the predator pressure classes defined in Figure 23. Not surprisingly, when there are large numbers of RIFA on the host plant, predator pressure is high (Figure 25).

It is possible that RIFA prey on monarch eggs or larvae opportunistically. High monarch mortality is expected when RIFA are present in high numbers and causing high predator pressure. The data were therefore divided into four classes according to low and high abundances of RIFA and low and high predator pressure. For this analysis, predator pressure classes were created by combining the first two classes in Figure 23 into low predator pressure and the second two classes in Figure 23 into high predator pressure. Two RIFA abundance classes were created: low RIFA abundance based on the first two classes in Figure 20B, and high RIFA abundance based on the last class in Figure 20B. The expectation was that when predator pressure is high due to high RIFA abundance, then monarch survival should be very low. However, this was not the case (Table 23). Monarch survival was not low when high predator pressure was due to high numbers of RIFA. In fact, though the trend was not significant, monarch survival was very high when there was both high predator pressure and high numbers of RIFA on the host plant. The failure of RIFA to opportunistically prey on monarchs in this context explains why there is no correlation between RIFA abundance on the host plants and monarch mortality.

Table 21. Summary of stepwise multiple regression analysis of RIFA number on control host plants based on non-predatory arthropod groups found on host plants that held at least one non-predatory arthropod. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.90 and significance level for removal from the model set at 0.80. Best model based on AICc.

Model	AICc	$_{\Delta}$AICc	w_i	F-Test	Model Probability
Aphids, Curculionidae, Leafhoppers, Chrysomelidae	-33.371	0.000	0.458	214.4500	<0.0001
Aphids, Curculionidae, Leafhoppers, Chrysomelidae, Other Milkweed Herbivores	-31.903	1.467	0.220	171.4600	<0.0001
Aphids, Curculionidae, Leafhoppers	-31.649	1.722	0.194	282.1300	<0.0001
Aphids, Curculionidae, Leafhoppers, Chrysomelidae, Other Milkweed Herbivores, Dermestidae	-30.170	3.201	0.092	142.6500	<0.0001
Aphids, Curculionidae, Leafhoppers, Chrysomelidae, Other Milkweed Herbivores, Dermestidae, Diptera < 5 mm in length	-28.254	5.117	0.035	121.9800	<0.0001

Table 22. Summary of the best fit model using stepwise multiple regression to predict the abundance of RIFA on control host plants based on the abundance of non-predatory arthropod groups.

Parameter	DF	Estimate	Standard Error	t-value	Pr > ChiSq
Intercept	1	-0.005001	0.041078	-0.12	0.9032
Aphids	1	0.022876	0.000847	27.01	<0.0001
Curculionidae	1	0.746097	0.064257	11.61	<0.0001
Chrysomelidae	1	-0.326564	0.168333	-1.94	0.0533
Leafhoppers	1	0.615406	0.215957	2.85	0.0047

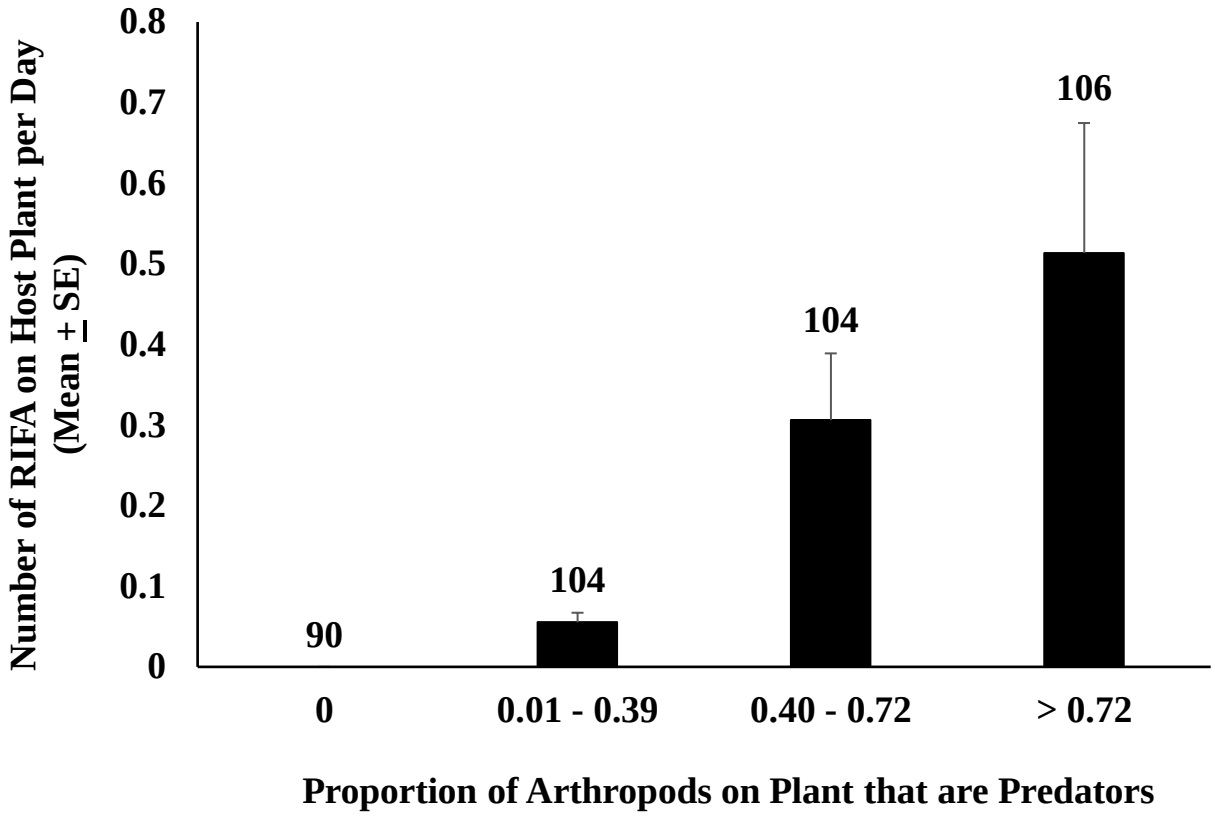

Figure 25. Relationship between the number of RIFA on control host plants and the predator pressure on those host plants. Host plants with high numbers of RIFA on them had high predator pressure (ANOVA, $F = 6.24$, $df = 3, 400$, $p = 0.0004$). Numbers over bars represent sample sizes (number of monarch eggs).

Table 23. Percent survival of monarch eggs and larvae relative to predator pressure and RIFA abundance on control host plants. Highlighted cell illustrates that monarch survival was not low when high predator pressure was caused by large numbers of RIFA on the host plant. Fisher's Exact Probability, p = 0.277.

	Predator Pressure (Proportion of Arthropods that are Predators)	
RIFA Abundance (Ind/day)	< 0.33	> 0.33
< 0.20 per day	18.2% (n = 181)	11.2% (n = 161)
> 0.20 per day	7.7% (n = 13)	16.33% (n = 49)

e. The Terrestrial Arthropod Community Surrounding Host Plants

The arthropod community surrounding host plants was measured using unbaited glue traps placed around the host plants. In 2017, data were collected from glue traps associated with 271 monarch eggs on 189 host plants. In 2018, data were collected from glue traps associated with 367 monarch eggs on 220 host plants. During the study 51,130 individuals from 86 arthropod groups were caught (Appendix 3). There were 34 predatory types of arthropods, including RIFA, the most abundant species captured. Flies (Diptera), aphids (Hemiptera – Aphoidea), mites (Acari), and small wasps (Hymenoptera – Apocrita) and leafhoppers (Hemiptera – Cicadellidae), had frequencies of 70% or higher. However, 27 groups of arthropods, about 1/3 of all taxa, were present at less than 10% of focal eggs or larvae. To overcome this sparse data, data were combined into 23 groups with minimum percent frequency of 21.7% (Appendix 4). A comparison of the communities observed on the plants (Appendices 1, 2) with those in the traps (Appendices 3,4) indicate marked differences. This, suggests that these communities are at least partly independent, probably because volant arthropods are more likely to occur on the plant than in the traps. Since gluing mealworms on to host plants was not expected to alter the surrounding terrestrial community, the data from RIFA enhanced host plants were not included in the analyses of trap data. The effective number of species and evenness of the terrestrial arthropod communities differed little between control plants and RIFA suppressed plants (Figure 26). Arthropods captured adjacent to control plants were compared to those captured adjacent to host plants in the RIFA enhanced treatment (Table 24). Aside from differences in the number of RIFA captured in the traps, there were significant differencesamong other groups of arthropods. Suppression of RIFA was associated with increased numbers of small flies, Isopods, Weevils (Curculionidae), grasshoppers and katydids (Caelifera and Tettigoniidae), small beetles, and Calyptrate flies (Table 24). Suppression of RIFA was also associated with decreased numbers of little black ants (*Monomorium minimum*) and millipedes (Table 24).

The abundance of arthropods captured in glue traps adjacent to control and RIFA suppressed monarch host plants was compared for host plants upon which larvae died prior to the third instar and host plants upon which the larvae survived to the third instar (Table 25). There were few differences

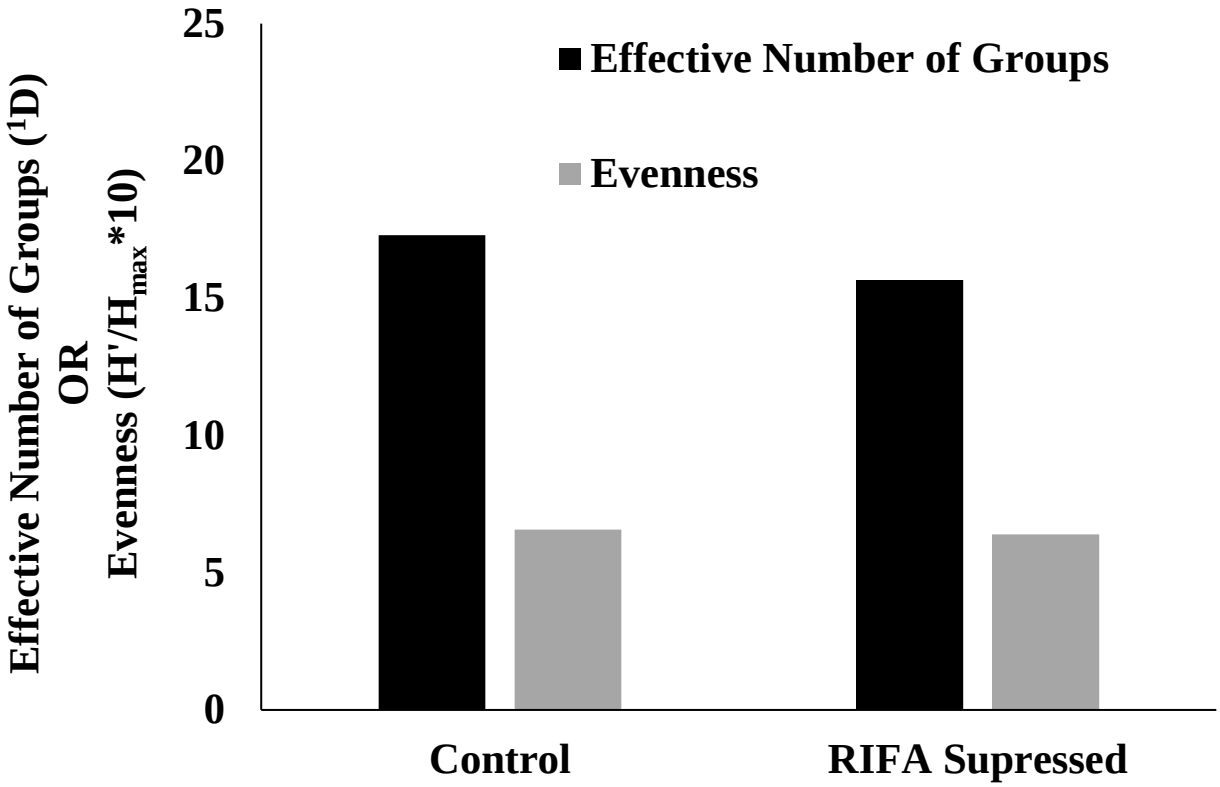

Figure 26. Effective number of arthropod groups and evenness of predominantly terrestrial arthropod communities captured in glue traps adjacent to host plants relative to treatment. Effective number of arthropod groups and community evenness were calculated using the Shannon Entropy Index.

Table 24. Abundances of arthropods (mean ± standard error) captured in glue traps adjacent to monarch host plants compared among control and RIFA suppressed treatments. Significant differences are highlighted in yellow and were assessed using Kruskal-Wallis ANOVA. See Appendix 4 for description of Arthropod groupings.

Arthropod Type	Control Mean ± SE (n = 194)	RIFA Suppressed Mean ± SE (n = 214)	Kruskal-Wallis Test X^2 (df =1)	P-value
Formicidae, *Solenopsis invicta*	1.067 + 0.103	0.078 + 0.010	243.1481	<0.0001
Diptera < 5 mm	0.634 + 0.042	0.803 + 0.051	6.7260	0.0095
Custacea: Isopoda	0.409 + 0.054	1.027 + 0.102	33.8132	<0.0001
Hemiptera, Aphididae	0.456 + 0.034	0.560 + 0.044	1.2223	0.2689
Arachnida, Acari	0.310 + 0.033	0.234 + 0.021	3.8112	0.0509
Thrips (Thysanoptera)	0.269 + 0.027	0.336 + 0.034	0.1243	0.7244
Formicidae, *Monomorium minimum*	0.245 + 0.045	0.022 + 0.005	33.7638	<0.0001
Hymenoptera, Apocrita < 5 mm	0.219 + 0.028	0.147 + 0.020	0.7431	0.3887
Lycosidae, Agelenidae, Pisuridae	0.117 + 0.011	0.152 + 0.017	3.0650	0.0800
Auchenorrhyncha	0.108 + 0.010	0.117 + 0.013	0.4977	0.4805
Other Predators	0.120 + 0.011	0.125 + 0.011	0.1669	0.6829
Orthoptera – Gryllidae	0.056 + 0.007	0.062 + 0.010	0.0108	0.9172

Table 24 Continued. Abundances of arthropods (mean ± standard error) captured in glue traps adjacent to monarch host plants compared among control and RIFA suppressed treatments. Significant differences are highlighted in yellow and were assessed using Kruskal-Wallis ANOVA. See Appendix 4 for description of Arthropod groupings.

Arthropod Type	Control	RIFA Suppressed	Kruskal-Wallis Test X^2 (df =1)	P-value
	Mean ± SE (n = 211)	Mean ± SE (n = 224)		
All Other Arthropods	0.083 + 0.010	0.091 + 0.008	1.9884	0.1585
Araneae, Others	0.083 + 0.008	0.085 + 0.007	0.8044	0.3698
Coleoptera, Curculionidae	0.069 + 0.006	0.104 + 0.008	8.9941	0.0027
Millipedes (Diplopoda)	0.151 + 0.028	0.032 + 0.009	32.5209	<0.0001
Calyptrate Flies	0.053 + 0.007	0.066 + 0.006	4.8284	0.0280
Araneae < 5 mm	0.068 + 0.007	0.063 + 0.005	0.4149	0.5195
Scavenging Beetles	0.070 + 0.007	0.051 + 0.005	2.4736	0.1158
Orthoptera, Caelifera and Tettigoniidae	0.033 + 0.004	0.073 + 0.007	19.9314	<0.0001
Coleoptera, < 10 mm	0.027 + 0.004	0.039 + 0.004	4.0139	0.0451
Harvestmen (Opiliones)	0.026 + 0.005	0.036 + 0.009	0.6200	0.4310
Chrysomelidae	0.024 + 0.004	0.033 + 0.005	2.5639	0.1093

between the trap captures associated with these host plants. However, host plants upon which monarch larvae survived to the third instar held more small spiders < 5 mm in body length (Table 24).

Logistic regression was used to develop a model to predict monarch survival based on the trap data. The top five of nine models are shown in Table 26. The best fit model included six groups of arthropods (Table 27). This model found that monarch survival was positively associated with the number small spiders and mites and was negatively associated with the number of RIFA, Isopods, other spiders, and harvestmen. All of the models included a negative influence of RIFA on monarch survival. However, none of these models are strong predictors of monarch survival, and the r-square value for the best fit model suggests that this model explains only 4% of the variation in the data. Furthermore, in the best fit model, the parameter estimate for RIFA is not statistically significant (Table 27).

The preceding analysis suggests that RIFA might play a role in monarch survival based on their presence in the surrounding community. However, because the two years of this study differed in terms of weather conditions and arthropod abundances on the host plants (see Table 10) the analyses were broken down by year. Comparisons were made of the number of individuals in each arthropod group for captures associated with control and RIFA suppressed host plants in each year (Table 28). Almost all of the arthropod groups varied in abundance between years. The exceptions were aphids, leaf hoppers, other predators, millipedes, and Chrysomelid beetles which changed very little between years. Of the remaining 18 groups, 15 groups of arthropods showed dramatically reduced populations in 2018 when compared to 2017. Three groups, Isopods, weevils, and calyptrate flies, showed increased abundances in 2018 when compared to 2017.

The analyses of survivorship relative to arthropods captured in traps adjacent to host plants were stratified by year. For the 2017 data, when arthropods were most abundant, the stepwise logistic regression procedure identified five models that predicted monarch survival (Table 29). None of these included RIFA. The best model included three arthropod groups, none of which had statistically significant parameter estimates. Wolf/grass/and nursery web spiders exhibited a positive, albeit weak,

Table 25. Mean (± Standard Error) abundance of arthropods captured in glue traps adjacent to monarch host plants where larvae survived to the third instar and adjacent to monarch host plants where larvae did not survive to the third instar. Data includes control and RIFA suppressed treatments. Significant differences are highlighted in yellow. For description of arthropod groups see Appendix 4.

Arthropod Type	Abundance Adjacent to Host Plants where Larvae Died (n = 338)	Abundance Adjacent to Host Plants where Larvae Survived (n = 70)	Kruskal-Wallis Test X^2 (df =1)	P-value
Formicidae, *Solenopsis invicta*	0.581 ± 0.065	0.392 ± 0.066	0.1474	0.7011
Diptera < 5 mm	0.699 ± 0.035	0.835 ± 0.104	1.1827	0.2768
Custacea: Isopoda	0.779 ± 0.071	0.511 ± 0.095	0.8131	0.3672
Hemiptera, Aphididae	0.520 ± 0.031	0.466 ± 0.063	0.7620	0.3827
Arachnida, Acari	0.253 ± 0.020	0.350 ± 0.060	2.8442	0.0917
Thrips (Thysanoptera)	0.301 ± 0.024	0.318 ± 0.056	0.0150	0.9026
Formicidae, *Monomorium minimum*	0.133 ± 0.026	0.104 ± 0.029	2.9205	0.0875
Hymenoptera, Apocrita < 5 mm	0.181 ± 0.019	0.182 ± 0.028	3.1334	0.0767
Lycosidae, Agelenidae, Pisuridae	0.126 ± 0.008	0.178 ± 0.047	0.2555	0.6132
Auchenorrhyncha	0.110 ± 0.009	0.124 ± 0.025	0.2818	0.5955
Other Predators	0.122 ± 0.008	0.124 ± 0.020	0.1845	0.6675
Orthoptera – Gryllidae	0.060 ± 0.007	0.056 ± 0.012	0.4983	0.4802

Table 25 Continued. Mean (± Standard Error) abundance of arthropods captured in glue traps adjacent to monarch host plants where larvae survived to the third instar and adjacent to monarch host plants where larvae did not survive to the third instar. Data includes control and RIFA suppressed treatments. Significant differences are highlighted in yellow. For description of arthropod groups see Appendix 4.

Arthropod Type	Abundance Adjacent to Host Plants where Larvae Died (n = 338)	Abundance Adjacent to Host Plants where Larvae Survived (n = 70)	Kruskal-Wallis Test X^2 (df =1)	P-value
All Other Arthropods	0.087 ± 0.007	0.084 ± 0.012	0.0023	0.9619
Araneae, Others	0.087 ± 0.006	0.072 ± 0.010	0.4611	0.4971
Coleoptera, Curculionidae	0.087 ± 0.006	0.092 ± 0.011	0.4685	0.4937
Millipedes (Diplopoda)	0.085 ± 0.016	0.108 ± 0.031	1.0448	0.3067
Calyptrate Flies	0.064 ± 0.005	0.042 ± 0.007	1.4972	0.2211
Araneae < 5 mm	0.060 ± 0.005	0.091 ± 0.012	6.5457	0.0105
Scavenging Beetles	0.059 ± 0.005	0.064 ± 0.009	1.1543	0.2826
Orthoptera, Caelifera and Tettigoniidae	0.054 ± 0.005	0.054 ± 0.010	0.0018	0.9663
Coleoptera, < 10 mm	0.033 ± 0.003	0.034 ± 0.007	0.0145	0.9042
Harvestmen (Opiliones)	0.034 ± 0.007	0.017 ± 0.005	0.5255	0.4685
Chrysomelidae	0.029 ± 0.004	0.026 ± 0.006	0.0332	0.8616

Table 26. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found in glue traps adjacent to control and RIFA suppressed host plants. Significance level for entry into the model was 0.30 and significance level for removal from the model set at 0.35.

Model	AICc	$_{\Delta}$AIC	w_i	Likelihood Ratio X^2	Model Probability
Araneae < 5 mm, RIFA, Acari, Calyptratae, Opiliones, Araneae (Others)	367.100	0.000	0.272	21.2870	0.0016
Araneae < 5 mm, RIFA, Acari, Calyptratae, Opiliones	367.234	0.134	0.254	19.0717	0.0019
Araneae < 5 mm, RIFA, Acari, Calyptratae, Opiliones, Araneae (Others), Isopoda	367.555	0.455	0.216	22.9231	0.0018
Araneae < 5 mm, RIFA, Acari, Calyptratae, Opiliones, Araneae (Others), Isopoda, Thysanoptera	368.529	1.429	0.133	24.0505	0.0022
Araneae < 5 mm, RIFA, Acari, Calyptratae	368.658	1.559	0.125	15.5763	0.0036

Table 27. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups caught in traps adjacent to control and RIFA enhanced host plants. Concordance of this model was 67.2%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > X^2
Intercept	1	-1.4819	0.2445	36.7339	<.0001
RIFA	1	-0.3484	0.209	2.7775	0.0956
Acari	1	0.474	0.2952	2.5785	0.1083
Araneae (Others)	1	-2.1836	1.5383	2.0149	0.1558
Calyptratae	1	-2.7537	1.9211	2.0547	0.1517
Araneae < 5 mm[a]	1	4.5376	1.4789	9.4141	0.0022
Opiliones[a]	1	-4.2902	2.7694	2.3998	0.1213

[a]Confidence intervals for these groups approached infinity.

Table 28. Mean (± Standard Error) abundance of arthropods captured in glue traps adjacent to control and RIFA enhanced host plants compared among years. This data includes both control and RIFA enhanced treatments. Significant differences are highlighted in yellow. For description of arthropod groups see Appendix 4.

Arthropod Type	Abundance Adjacent to Host Plants in 2017 (n = 184)	Abundance Adjacent to Host Plants in 2018 (n = 224)	Kruskal-Wallis Test X^2 (df =1)	P-value
Formicidae, *Solenopsis invicta*	0.6569 + 0.0708	0.4590 + 0.0808	11.3069	0.0008
Diptera < 5 mm	1.1552 + 0.0543	0.3672 + 0.0234	163.7500	<0.0001
Custacea: Isopoda	0.2582 + 0.0363	1.1228 + 0.1003	73.6650	<0.0001
Hemiptera, Aphididae	0.4623 + 0.0379	0.5502 + 0.0407	3.1950	0.0739
Arachnida, Acari	0.3339 + 0.0291	0.2174 + 0.0253	21.9615	<0.0001
Thrips (Thysanoptera)	0.5214 + 0.0400	0.1253 + 0.0158	91.9050	<0.0001
Formicidae, *Monomorium minimum*	0.2238 + 0.0461	0.0491 + 0.0132	22.3630	<0.0001
Hymenoptera, Apocrita < 5 mm	0.3047 + 0.0341	0.0801 + 0.0072	60.8856	<0.0001
Lycosidae, Agelenidae, Pisuridae	0.2425 + 0.0198	0.0466 + 0.0044	179.6095	<0.0001
Auchenorrhyncha	0.0829 + 0.0065	0.1373 + 0.0138	3.5384	0.0600
Other Predators	0.1189 + 0.0102	0.1256 + 0.0114	0.0798	0.7775
Orthoptera – Gryllidae	0.1182 + 0.0121	0.0106 + 0.0019	131.4348	<0.0001

Table 28 Continued. Mean (± Standard Error) abundance of arthropods captured in glue traps adjacent to control and RIFA enhanced host plants compared among years. This data includes both control and RIFA enhanced treatments. Significant differences are highlighted in yellow. For description of arthropod groups see Appendix 4.

Arthropod Type	Abundance Adjacent to Host Plants in 2017 (n = 184)	Abundance Adjacent to Host Plants in 2018 (n = 224)	Kruskal-Wallis Test X^2 (df =1)	P-value
All Other Arthropods	0.1080 + 0.0106	0.0695 + 0.0068	16.7597	<0.0001
Araneae, Others	0.0992 + 0.0086	0.0717 + 0.0055	4.1289	0.0422
Coleoptera, Curculionidae	0.0696 + 0.0057	0.1021 + 0.0077	5.1377	0.0234
Millipedes (Diplopoda)	0.0499 + 0.0117	0.1208 + 0.0248	1.7447	0.1865
Calyptrate Flies	0.0401 + 0.0053	0.0762 + 0.0072	19.2957	<0.0001
Araneae < 5 mm	0.1165 + 0.0073	0.0234 + 0.0029	131.0103	<0.0001
Scavenging Beetles	0.0808 + 0.0074	0.0424 + 0.0043	15.5003	<0.0001
Orthoptera, Caelifera and Tettigoniidae	0.0673 + 0.0061	0.0427 + 0.0059	21.5931	<0.0001
Coleoptera, < 10 mm	0.0537 + 0.0049	0.0165 + 0.0029	50.3656	<0.0001
Harvestmen (Opiliones)	0.0496 + 0.0116	0.0159 + 0.0026	4.6700	0.0307
Chrysomelidae	0.0197 + 0.0028	0.0360 + 0.0055	2.2154	0.1366

Table 29. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found in glue traps adjacent control and RIFA enhanced host plants in 2017. Significance level for entry into the model was 0.30 and significance level for removal from the model set at 0.35.

Model	AICc	$_{\Delta}$AIC	w_i	Likelihood Ratio X^2	Model Probability
Wolf/Grass/Nursery Web Spiders, All other Predators, Araneae < 5 mm	172.392	0.000	0.223	9.0535	0.0286
Wolf/Grass/Nursery Web Spiders, All other Predators	172.489	0.097	0.212	6.8426	0.0327
Wolf/Grass/Nursery Web Spiders	172.493	0.101	0.212	4.7488	0.0293
Wolf/Grass/Nursery Web Spiders, All other Predators, Araneae < 5 mm, Diptera < 5 mm	172.656	0.263	0.195	10.9275	0.0274
Wolf/Grass/Nursery Web Spiders, All other Predators, Araneae < 5 mm, Diptera < 5 mm, Aphids	173.084	0.692	0.158	12.6599	0.0268

Table 30. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups caught in traps adjacent to control and RIFA enhanced host plants in 2017. Concordance of this model was 59.9%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > X^2
Intercept	1.000	-1.878	0.3713	25.5882	<0.0001
Wolf/Grass/Nursery Web Spiders	1.000	1.121	0.6781	2.7345	0.0982
All other Predators	1.000	-3.044	1.9167	2.5218	0.1123
Araneae < 5 mm[a]	1.000	3.022	1.9967	2.2909	0.1301

[a]Confidence intervals for these groups approached infinity.

effect on monarch survival (Table 30). The 2018 data yielded fourteen models that fit the selection criteria. The five best models are shown in Table 31, none of which included RIFA as important effects on monarch survival. The best model included ten arthropod groups (Table 32). In this model, wolf/grass/and nursery web spiders, Isopods, Thrips, Calyptrate flies and harvestmen negative impacts on monarch survival. Small flies, other predators, crickets, other non-predatory arthropods, and small spiders all had a positive impact on monarch survival. The concordance of this model is high (79.8%), suggesting a good fit to the data. However, six of the ten parameter estimates had confidence intervals that approach infinity suggesting that these estimates are inflated.

The conclusion is that terrestrial arthropod populations surrounding host plants varied markedly among years and the number and types of arthropods influencing monarch survival also varied among years. In the year when the abundances of arthropods were lower, a greater number of arthropods are implicated as affecting monarch survival. Overall, higher abundances of RIFA in the terrestrial arthropod community surrounding host plants had a negative impact on monarch survival. However, this effect was very small and, when the data are analyzed separately for each year, or if only control plants are included, this effect disappears.

f. Host Plant Phenotype and Condition

Host plant attributes were measured as described in the methods section. For analytical purposes these analyses were restricted to control plants only. In addition, plants whose ramets were completely browsed were eliminated. This left 398 plants for analysis.

Host plants were evaluated relative to their size, number of leaves, and number of ramets (Table 33). The average *A. viridis* host plant had just over two ramets, a total ramet length of over 60 cm, over 34 adult leaves, and a cardenolide concentration of 0.338 mg/0.1g. Host plants often had pathological traits (see methods) (Table 33, Table 34). Most plants exhibited some extent of leaf curling and most plants (65.8%) exhibited at least one of the pathological symptoms shown in Table 34. Over half of the

Table 31. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups found in glue traps adjacent to control and RIFA enhanced host plants in 2018. Significance level for entry into the model was 0.30 and significance level for removal from the model set at 0.35.

Model	AICc	$_{\Delta}$AIC	w_i	Likelihood Ratio X^2	Model Probability
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones, Diptera < 5 mm, Other Predators, Calyptratae, Other non-predatory Arthropods, Thrips	180.399	0.000	0.283	43.8549	<0.0001
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones, Diptera < 5 mm, Other Predators, Calyptratae, Other Non-predatory Arthropods, Thrips, Scavenging Beetles	180.967	0.569	0.213	45.5411	<0.0001
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones, Diptera < 5 mm, Other Predators, Calyptratae, Other non-predatory Arthropods	181.308	0.910	0.179	40.7114	<0.0001
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones, Diptera < 5 mm, Other Predators, Calyptratae	181.364	0.965	0.174	38.4442	<0.0001
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones	181.654	1.255	0.151	31.6403	<0.0001
Araneae < 5 mm, Wolf/Grass/Nursery Web Spiders, Crickets, Isopods, Opiliones, Diptera < 5 mm, Other Predators, Calyptratae, Other non-predatory Arthropods, Thrips	180.399	0.000	0.283	43.8549	<0.0001

Table 32. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on arthropod groups caught in traps adjacent to control and RIFA enhanced host plants in 2018. Concordance of this model was 79.8%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > X^2
Intercept	1	-1.7356	0.4232	16.8198	<.0001
Diptera < 5 mm	1	1.3038	0.6349	4.2175	0.04
Isopods	1	-0.3932	0.1754	5.0285	0.0249
Thrips	1	-1.8022	1.1842	2.316	0.1281
Wolf/Grass/Nursery Web Spiders[a]	1	-11.8637	4.6725	6.4467	0.0111
Other Predators	1	1.6936	1.0528	2.5875	0.1077
Crickets[a]	1	12.1768	6.1847	3.8764	0.049
Other Non-predatory Arthropods[a]	1	4.0427	2.6152	2.3896	0.1221
Calyptratae[a]	1	-6.4016	3.3891	3.5679	0.0589
Araneae < 5 mm[a]	1	17.4006	4.5268	14.7758	0.0001
Opiliones[a]	1	-17.8863	10.1532	3.1034	0.0781

[a]Confidence intervals for these groups approached infinity.

Table 33. General physical characteristics of 398 host plants.

	Mean ± Standard Error
Number of Ramets	2.324 ± 0.080
Total Ramet Length	64.820 ± 2.658
Total Number of Mature Leaves	34.466 ± 1.302
Total Cardenolides (mg/0.1g) (n = 550)	0.338 ± 0.006
Curling Score	2.426 ± 0.052

Table 34. Pathological attributes associated with 398 host plants.

	Number of Plants with Symptoms	Number of Plants without Symptoms	Percent Affected
Herbivory	231	167	58.04
Yellowing	144	254	36.18
Leaf Spotting	120	278	30.15
Darkening of Veins	72	326	18.09
Darkening of Leaf Not Including Veins	69	329	17.34
Leaf Miners	66	332	16.58
General Necrosis	65	333	16.33
Shoot Tip Necrosis	52	346	13.07
General Wilt	37	361	9.30
Browsed	29	369	7.29
Stem Weevil Damage	20	378	5.03

plants showed obvious signs of herbivory. The other most common pathologies were yellowing and leaf spotting, each affecting over 20% of host plants. The remaining traits were uncommon (Table 34).

There is no information on the diseases and pathology of *A. viridis* and their causal agents. Leaf curling was attributed to heat or water stress. General wilt and general necrosis were attributed to thermal stress, water stress, wind damage, and other sources of physical damage such as trampling. Herbivory, leaf miners, shoot tip necrosis, and stem weevil damage were all attributable to arthropods. Browsing was most likely due to rabbits. To determine what might cause the remaining traits, 10 samples each of plants exhibiting yellowing, leaf spotting, darkening of leaf blades, and darkening of leaf veins were sent for analyses of soil characteristics and plant pathogens (see methods). The soil characteristics associated with each of these traits are shown in Tables 35 through 38. The nutrients and minerals associated with control plants indicate soils that are rather deficient. Comparisons of soil parameters associated with control plants and affected plants revealed a few significant differences. However, in each of these cases the affected plants had higher levels of the parameter. Yellowing was associated with elevated levels of phosphorus, potassium, zinc, copper and electrical conductivity (Table 35). Darkening of the leaf veins was associated with elevated levels of phosphorus and copper (Table 36). Darkening of the leaf blades was associated with elevated levels of phosphorus, potassium, and zinc (Table 37). Leaf spotting was associated with elevated levels of phosphorus and copper (Table 38). Since none of these parameters are high enough to cause pathological symptoms, it is unlikely that these differences were directly causal to the symptoms observed. It is more likely that these results are due to small sample sizes and a resulting bias among the control plants that drove these differences.

Ten samples each of plants exhibiting yellowing, leaf spotting, darkening, and vein darkening were screened for common viral and fungal pathogens. These included Cucumber Mosaic Virus, Tomato Spotted Wilt Virus, Impatiens Necrotic Spot Virus, Potyvirus, Rhizoctonia, and Crown Rust. Potyvirus was the most common pathogen found and it occurred even in some asymptomatic controls (Table 39).

Table 35. Soil parameters associated with yellowing of *A. viridis* leaves.

Parameter	Yellowing Mean ± SE	Control Mean ± SE	F (df = 1, 17)	p
P	2.09 ± 0.17	1.03 ± 0.21	15.09	0.0012
K	178.64 ± 9.44	128.40 ± 6.22	20.54	0.0003
Ca	10467.66 ± 486.95	9237.48 ± 952.83	1.23	0.2822
Mg	378.61 ± 20.64	416.08 ± 18.54	1.83	0.1934
S	4.21 ± 0.51	3.66 ± 0.65	0.43	0.5199
Fe	6.46 ± 0.92	5.85 ± 0.79	0.25	0.6204
Mn	1.42 ± 0.19	1.82 ± 0.40	0.75	0.3989
Zn	0.15 ± 0.04	0.05 ± 0.01	6.37	0.0219
Cu	0.24 ± 0.02	0.18 ± 0.02	4.62	0.0462
pH	7.67 ± 0.15	7.59 ± 0.17	0.12	0.7310
EC	424.56 ± 26.22	319.40 ± 24.24	8.70	0.0090

Table 36. Soil parameters associated with darkening of the veins of *A. viridis* leaves.

	Darkened Veins	Control		
Parameter	Mean ± SE	Mean ± SE	F (df = 1, 19)	p
P	1.79 ± 0.20	1.03 ± 0.21	6.87	0.0168
K	148.08 ± 9.38	128.40 ± 6.22	2.93	0.1032
Ca	9270.81 ± 631.77	9237.48 ± 952.83	0.00	0.9766
Mg	429.34 ± 26.42	416.08 ± 18.54	0.16	0.6914
S	4.09 ± 0.22	3.66 ± 0.65	0.42	0.5241
Fe	7.78 ± 1.14	5.85 ± 0.79	1.87	0.1874
Mn	1.48 ± 0.23	1.82 ± 0.40	0.58	0.4572
Zn	0.08 ± 0.01	0.05 ± 0.01	2.99	0.1001
Cu	0.22 ± 0.01	0.18 ± 0.02	4.65	0.0440
pH	7.55 ± 0.17	7.59 ± 0.17	0.03	0.8679
EC	320.82 ± 27.03	319.40 ± 24.24	0.00	0.9695

Table 37. Soil parameters associated with darkening of the blades of *A. viridis* leaves.

Parameter	Darkened Leaf Blades Mean ± SE	Control Mean ± SE	F (df = 1, 19)	p
P	1.83 ± 0.24	1.03 ± 0.21	6.41	0.0209
K	166.85 ± 13.14	128.40 ± 6.22	7.00	0.0165
Ca	11290.42 ± 915.62	9237.48 ± 952.83	2.41	0.1377
Mg	365.51 ± 20.11	416.08 ± 18.54	3.42	0.0810
S	4.36 ± 0.22	3.66 ± 0.65	1.02	0.3255
Fe	5.20 ± 0.88	5.85 ± 0.79	0.30	0.5880
Mn	1.57 ± 0.54	1.82 ± 0.40	0.14	0.7159
Zn	0.15 ± 0.02	0.05 ± 0.01	11.21	0.0036
Cu	0.22 ± 0.02	0.18 ± 0.02	2.05	0.1697
pH	7.83 ± 0.17	7.59 ± 0.17	1.03	0.3230
EC	340.20 ± 28.05	319.40 ± 24.24	0.31	0.5817

Table 38. Soil parameters associated with spotting of *A. viridis* leaves.

	Leaf Spotting	Control		
Parameter	Mean ± SE	Mean ± SE	F (df = 1, 19)	p
P	2.42 ± 0.43	1.03 ± 0.21	8.99	0.0081
K	143.13 ± 20.35	128.40 ± 6.22	0.52	0.4787
Ca	8152.33 ± 1242.31	9237.48 ± 952.83	0.49	0.4926
Mg	436.39 ± 22.99	416.08 ± 18.54	0.48	0.4971
S	4.53 ± 0.35	3.66 ± 0.65	1.30	0.2703
Fe	9.77 ± 2.01	5.85 ± 0.79	3.57	0.0759
Mn	2.13 ± 0.47	1.82 ± 0.40	0.24	0.6306
Zn	0.28 ± 0.14	0.05 ± 0.01	2.79	0.1130
Cu	0.23 ± 0.01	0.18 ± 0.02	5.43	0.0323
pH	7.38 ± 0.23	7.59 ± 0.17	0.58	0.4549
EC	280.27 ± 45.80	319.40 ± 24.24	0.60	0.4474

Table 39. Percent occurrence of plant pathogens associated with four common plant pathologies observed among *A. viridis* host plants. N = 10 for each plant pathology.

	Cucumber Mosaic Virus	Tomato Spotted Wilt Virus	Impatiens Necrotic Spot Virus	Potyvirus	*Rhizoctonia sp*	Crown Rust
Asymptomatic (Control)	0	0	0	20	10	0
Yellowing of Leaves	20	0	10	40	30	0
Leaf Spotting	0	0	0	100	10	10
Darkening of Leaves	0	0	20	100	0	0
Darkening of Leaf Veins	0	0	0	90	0	0

Leaf spotting, darkening of the leaf blades and veins all appear to be due to the presence of Potyvirus where the frequency of potyviruses was 90 to 100 percent (Table 39). Yellowing may be the result of multiple causes as it was associated with several pathogens and aphids (see below).

Since most plant viruses are vectored by arthropods (Whitfield et al. 2015), each trait was investigated relative to the presence or absence of milkweed-specialist herbivores (Tables 40 through 43). Though leaf spotting and darkening of both the leaf blades and the leaf veins are associated with Potyvirus, they are associated with different potential insect vectors. Leaf spotting is strongly associated with weevils, milkweed bugs, and thrips (Table 40). Darkening of the leaf blades is most strongly associated with milkweed bugs (Table 41). Darkening of the leaf veins is not associated with any of the potential vectors of Potyvirus (Table 42). These differences may reflect differences in the mode of infection (type of vector) or the relative stage of advancement of the disease. Yellowing appeared to be weakly associated with aphids (Table 43).

The purpose of measuring plant characteristics was to determine how they affected the plant arthropod community and monarch survival. Indices of trait intensity were calculated for leaf curling, darkening of the leaf blades, darkening of leaf veins, yellowing, spot fungus, and leaf miner damage using photographs. Because not all plants had photographs taken of their pathologies, it was not possible to calculate indices for all the plants in the data set. Therefore, the following analyses are based on plants associated with 339 eggs. Also excluded from these analyses were the insects identified in Tables 39 through 42 as potential vectors of plant pathogens.

The potential response of the remaining arthropod groups was modeled using stepwise multiple regression, wherein corrected Aikaike's Information Criterion (AICc) was used to select the best combination of plant attributes explaining the presence of an arthropod group on host plants. Validity of individual parameters within these models was evaluated based on t-tests using $\alpha = 0.05$ as the rejection

Table 40. Percent occurrence of milkweed-feeding insects relative to the expression of leaf spotting (n = 398 host plants).

	Symptomatic Plants (n = 120)	Asymptomatic Plants (n = 278)	Chi-square[a] (df = 1)	p
Weevils (Curculionidae) (n=82)	35.00	14.39	21.7685	<0.0001
Milkweed Bugs (Lygaeidae) (n=94)	34.17	19.06	10.5967	0.0011
Thrips (Thysanoptera) (n=25)	13.33	3.24		0.0004[b]
Aphids (Aphidoidea) (n=120)	27.50	31.29	0.5732	0.4490

[a] 2x2 Contingency tables

[b]Probability based on Fisher's Exact Test

Table 41. Percent occurrence of milkweed-feeding insects relative to the expression of darkening of leaf blades (n = 398 host plants).

	Symptomatic Plants (n = 69)	Asymptomatic Plants (n = 329)	Chi-square[a] (df = 1)	P
Weevils (Curculionidae) (n=82)	26.09	19.45	1.5346	0.2154
Milkweed Bugs (Lygaeidae) (n=94)	40.58	20.06	13.3118	0.0003
Thrips (Thysanoptera) (n=25)	7.25	6.08		0.7841[b]
Aphids (Aphidoidea) (n=120)	30.43	30.09	0.0032	0.9549

[a] 2x2 Contingency tables

[b]Probability based on Fisher's Exact Test

Table 42. Percent occurrence of milkweed-feeding insects relative to the expression of darkening of the leaf veins (n = 398 host plants).

	Symptomatic Plants (n = 72)	Asymptomatic Plants (n = 326)	Chi-square[a] (df = 1)	p
Weevils (Curculionidae) (n=82)	12.50	22.39	3.5282	0.0603
Milkweed Bugs (Lygaeidae) (n=94)	18.06	24.85	1.5077	0.2195
Thrips (Thysanoptera) (n=25)	2.78	7.06		0.2804[b]
Aphids (Aphidoidea) (n=120)	36.11	28.83	1.4828	0.2233

[a] 2x2 Contingency tables

[b]Probability based on Fisher's Exact Test

Table 43. Percent occurrence of milkweed-feeding insects relative to the expression of yellowing (n = 398 host plants).

	Symptomatic Plants (n = 144)	Asymptomatic Plants (n = 254)	Chi-square[a] (df = 1)	p
Weevils (Curculionidae) (n=82)	17.36	22.44	1.4497	0.2286
Milkweed Bugs (Lygaeidae) (n=94)	21.53	24.80	0.5465	0.4597
Thrips (Thysanoptera) (n=25)	9.03	4.72		0.1304[b]
Aphids (Aphidoidea) (n=120)	37.50	25.98	5.7868	0.0161

[a] 2x2 Contingency tables

[b]Probability based on Fisher's Exact Test

criterion. None of the plant attributes adequately explained the abundance of other ants on the host plants criterion. None of the plant attributes adequately explained the abundance of other ants on the host plants (Table 44). The dominant trend for the remaining arthropod groups was that arthropod abundance was predicted by attributes of the plant's physical size. In most cases, arthropods were more abundant on plants with either a greater total ramet length or a larger total number of leaves (Table 44). Both of these are associated with larger plants. Interestingly, most arthropod abundances were negatively associated with the total number of ramets (Table 44). The same trends occurred when groups are combined into all non-predatory arthopods, all predatory arthropods, and all arthropods on the host plant (Table 44). Two groups of arthropods, RIFA and Chrysomelid beetles, were found in higher abundance on host plants with higher levels of cardenolides. In only two cases were individual arthropod groups significantly associated with plant pathology. All other arthropods were more abundant on plants with greater levels of shoot-tip necrosis and mites were more abundant on plants with greater intensity of darkened leaf veins. The total number of non-predatory arthropods was higher on host plants with more intense expression of darkened leaf veins and was lower on host plants with a greater degree of leaf curling (Table 44). The conclusion from these analyses is that plant pathology does not have a large impact on the arthropod community on the host plants. However larger host plants harbor larger populations of arthropods.

Stepwise logistic regression was used to determine which plant attributes most predicted the survival of monarch eggs to the third instar. Sixteen models fit the selection criteria and the top five models are provided in Table 45. None of these models was particularly strong, and the best model contained four parameters, number of leaves, leaf curling, herbivory, and browse (Table 46). However, only two of these parameters were statistically significant. Monarch survival increased on plants with more ramets and a lower degree of leaf curling. Aside from these parameters, host plant attributes and pathology does not appear to have any impact on monarch survival to the third instar.

Table 44. Multiple regression models used to interpret the effect of plant characteristics and pathology on arthropod occupancy of 339 host plants. Selection criteria for best fit model was based on AICc.

Arthropod Group	Parameters Selected	Parameter Estimate	Parameter t-value	Parameter p-value	Model F-value	Model p-value
Little Black Ant (*Monomorium minimum*)	Number of Ramets	-0.309878	-2.57	0.0105	10.86	<0.0001
	Ramet Length	-0.248644	-2.24	0.0256		
	Number of Leaves	0.028841	4.53	<0.0001		
	Leaf Curling	-0.02405	-1.9	0.0589		
RIFA (*Solenopsis invicta*)	Ramet Length	0.001691	1.84	0.067	8.92	<0.0001
	Browsed	-1.282017	-1.6	0.1111		
	Cardenolides	1.919337	4.79	<0.0001		
Other Ants	No Models Fit Selection Criteria					
All Other Arthopods	Number of Ramets	-0.023066	-2.98	0.0031	13.85	<0.0001
	Ramet Length	-0.00081	-1.83	0.0685		
	Number of Leaves	0.004795	5.42	<0.0001		
	Leaf Curling	-0.011383	-1.43	0.154		
	Shoot Necrosis	0.049017	2.86	0.0045		
	Leaf Miners	22.057843	1.86	0.0635		

Table 44 Continued. Multiple regression models used to interpret the effect of plant characteristics and pathology on arthropod occupancy of 339 host plants. Selection criteria was based on AICc.

Arthropod Group	Parameters Selected	Parameter Estimate	Parameter t-value	Parameter p-value	Model F-value	Model p-value
Diptera < 5 mm in length	Number of Ramets	0.011234	1.63	0.1044	15.48	<0.0001
	Number of Leaves	0.001012	2.27	0.024		
	Browsed	0.201392	1.78	0.0759		
Other Predators Not Including Ants and Jumping Spiders	Number of Ramets	-0.014413	-2.15	0.0319	25.41	<0.0001
	Number of Leaves	0.002821	6.52	<0.0001		
	General Necrosis	-0.458284	-2.03	0.0434		
Jumping Spiders	Number of Ramets	-0.035042	-3.75	0.0002	15.65	<0.0001
	Ramet Length	0.002023	6.96	<0.0001		
	Leaf Curling	-0.018025	-1.85	0.0648		
	Darkening of leaf blades	-18.26878	-1.69	0.0913		
Unknown Beetles	Number of Ramets	-0.009888	-1.52	0.1291	2.25	0.1067
	Number of Leaves	0.000886	2.1	0.0365		

Table 44 Continued. Multiple regression models used to interpret the effect of plant characteristics and pathology on arthropod occupancy of 340 host plants. Selection criteria was based on AICc.

Arthropod Group	Parameters Selected	Parameter Estimate	Parameter t-value	Parameter p-value	Model F-value	Model p-value
Chrysomelidae	Number of Ramets	-0.02599	-3.49	0.0005	17.68	<0.0001
	Number of Leaves	0.003397	7.22	<0.0001		
	Leaf Curling	-0.020464	-2.64	0.0088		
	Herbivory	-0.168443	-1.53	0.1266		
	Cardenolides	0.135755	2.3	0.0221		
Mites	Number of Ramets	0.014018	3.48	0.0006	14.72	<0.0001
	Leaf Curling	0.010292	1.49	0.1367		
	Darkening of leaf veins	0.126876	5.42	<0.0001		
	Browsed	0.285059	2.72	0.0069		
Leafhoppers	Ramet Length	0.001017	8.73	<0.0001	20.71	<0.0001
	Leaf Curling	-0.011617	-1.87	0.062		
	Shoot Necrosis	-0.022132	-1.56	0.1195		
	Cardenolides	-0.096752	-1.97	0.0499		
Dermestid Beetles	Number of Ramets	-0.020129	-2.51	0.0126	4.24	0.0152
	Ramet Length	0.000755	2.9	0.004		

Table 44 Continued. Multiple regression models used to interpret the effect of plant characteristics and pathology on arthropod occupancy of 340 host plants. Selection criteria was based on AICc.

Arthropod Group	Parameters Selected	Parameter Estimate	Parameter t-value	Parameter p-value	Model F-value	Model p-value
All Non-Predators	Number of Ramets	-0.169665	-5.04	<.0001	38.03	<0.0001
	Ramet Length	0.007451	3.86	0.0001		
	Number of Leaves	0.009626	2.51	0.0126		
	Leaf Curling	-0.091022	-2.65	0.0084		
	Browsing	1.027601	1.97	0.0494		
All Predators	Number of Ramets	-0.54366	-2.62	0.0092	10.99	<0.0001
	Ramet Length	0.032639	4.77	<.0001		
	Herbivory	5.152316	1.6	0.1099		
All Arthropods	Number of Ramets	-0.712553	-3.35	0.0009	16.58	<0.0001
	Ramet Length	0.044311	6.31	<.0001		
	Herbivory	5.339065	1.62	0.1062		

Table 45. Summary of stepwise logistic regression analysis of survival of 339 monarch eggs or larvae based on physical attributes of the host plants. A stepwise selection procedure was used to generate these models with significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35.

Model	AICc	$_{\Delta}$AICc	w_i	Likelihood Ratio X^2	Model Probability
Number of Leaves, Leaf Curling, Herbivory, Browsed	290.547	0.000	0.251	13.6554	0.0085
Number of Leaves, Leaf Curling, Herbivory, Browsed, Ramet Length	290.897	0.350	0.211	15.3910	0.0088
Number of Leaves, Leaf Curling, Herbivory, Browsed, Ramet Length, Number of Ramets	291.094	0.547	0.191	17.2918	0.0083
Number of Leaves, Leaf Curling, Herbivory, Browsed, Ramet Length, Number of Ramets, Cardenolides	292.280	1.733	0.106	18.2171	0.0110
Number of Leaves, Leaf Curling, Herbivory	293.075	2.528	0.071	9.0545	0.0286

Table 46. Summary of the best fit model using logistic regression of survival of monarch eggs or larvae based on physical attributes of the host plants. Concordance of this model was 64.9%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > X^2
Intercept	1	-1.5028	0.4017	13.9937	0.0002
Number of Leaves	1	0.0133	0.00507	6.9066	0.0086
Leaf Curling	1	-0.3324	0.1613	4.2481	0.0393
Herbivory[1]	1	3.4538	1.9067	3.2812	0.0701
Browsed[1]	1	-9.4093	6.6883	1.9792	0.1595

[1] 95% Wald confidence interval of this parameter approached infinity, reflecting sparse data bias.

The average cardenolide concentration of host plants was 0.338 mg/0.1g (Table 33). For cardenolide analyses, leaves were collected from host plants and from adjacent plants that did not contain monarch eggs or larvae and that did not show evidence of herbivory. These adjacent plants were typically within 1 meter of the host plant. Plants upon which eggs were laid but did not hatch were considered to represent plants selected by females for oviposition. Adjacent, unoccupied plants next to plants selected by females for oviposition were considered to be plants that females did not chose for oviposition. There was a tendency for females to lay eggs on host plants that had lower cardenolide content than adjacent plants but this trend was not statistically significant (Figure 27). The cardenolide concentration of plants upon which monarch larvae survived to the third instar did not differ from the cardenolide concentration of plants upon which monarch larvae failed to reach the third instar (Figure 28).

Stepwise multiple regressions were used to determine whether any of the physical characteristics of the host plants were related to cardenolide content. This procedure identified 14 models that fit the selection criteria. Based on AIC selection, the best model contained eight variables (Table 47). In general, plants with more leaves and fewer ramets had higher levels of cardenolides. Plants with a greater total ramet length tend to have higher cardenolide concentration as do plant that are browsed. Plants with greater darkening of leaf blades and more leaf spotting and, therefore, infected with Potyvirus, also had higher concentrations of cardenolides. Lastly, plants that had been browsed by rabbits also had higher levels of cardenolides (Table 47).

g. Survivorship of fall monarch eggs and larvae in north Texas

Fall egg and larval survivorship was measured at a site near the city of Sulphur Spring, Hopkins Co., Texas, in the fall of 2017 (See Figure 10). Fourteen individuals were found as first instars and were eliminated from analyses. One individual was lost. Among the remaining 231 eggs, there were 9 individuals that survived long enough to be identified as Queens (*Danaus gilippus*), only one of which reached the third instar. Because queen eggs cannot be distinguished in the field from monarch eggs, both queen and monarch eggs are combined in the following analysis.

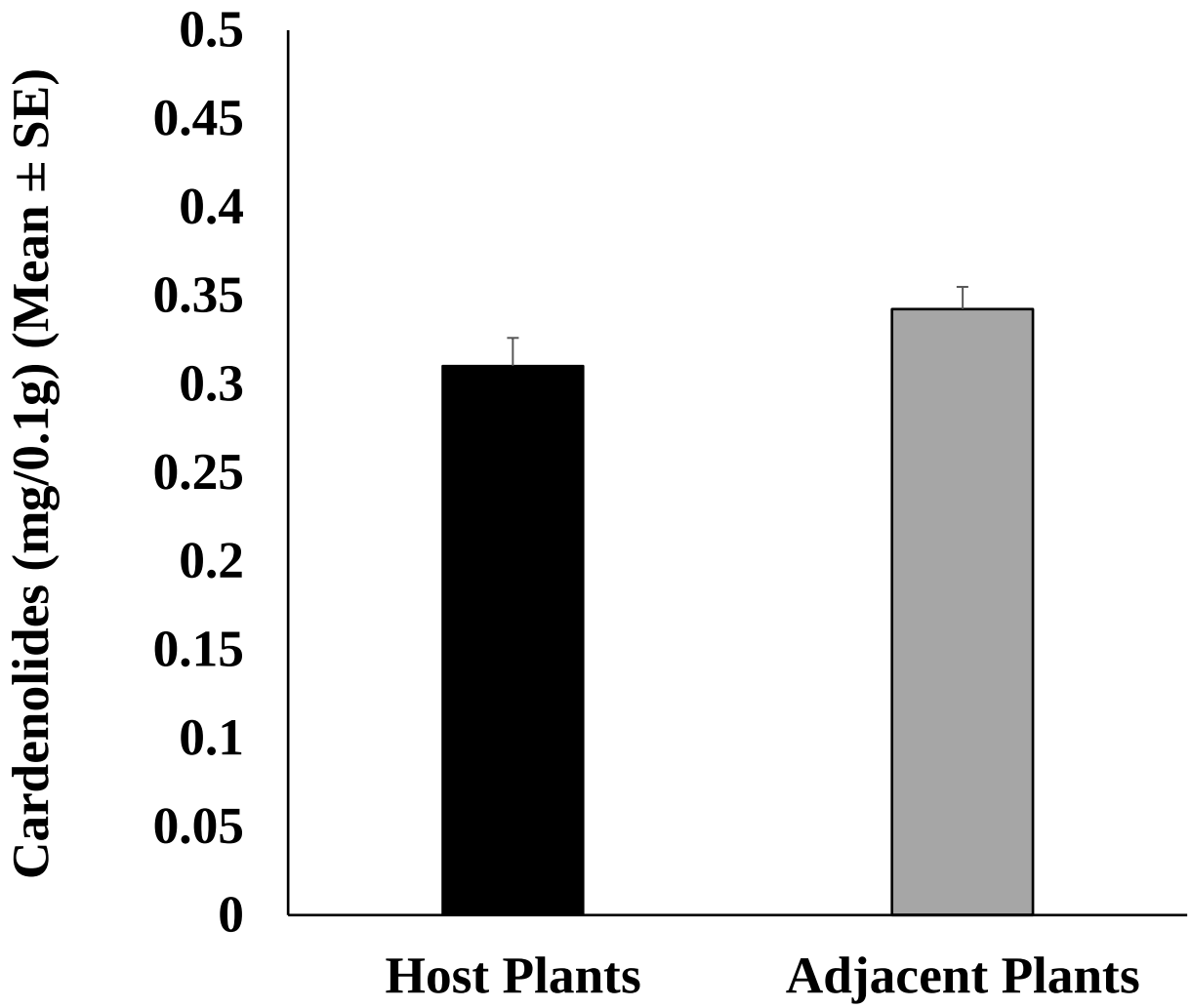

Figure 27. Mean cardenolide content of leaves for plants chosen by female monarch for oviposition and adjacent plants that were not chosen for oviposition. Paired data based on 24 host plants. Paired T-test; t = 1.81152, n = 83, p = 0.0737.

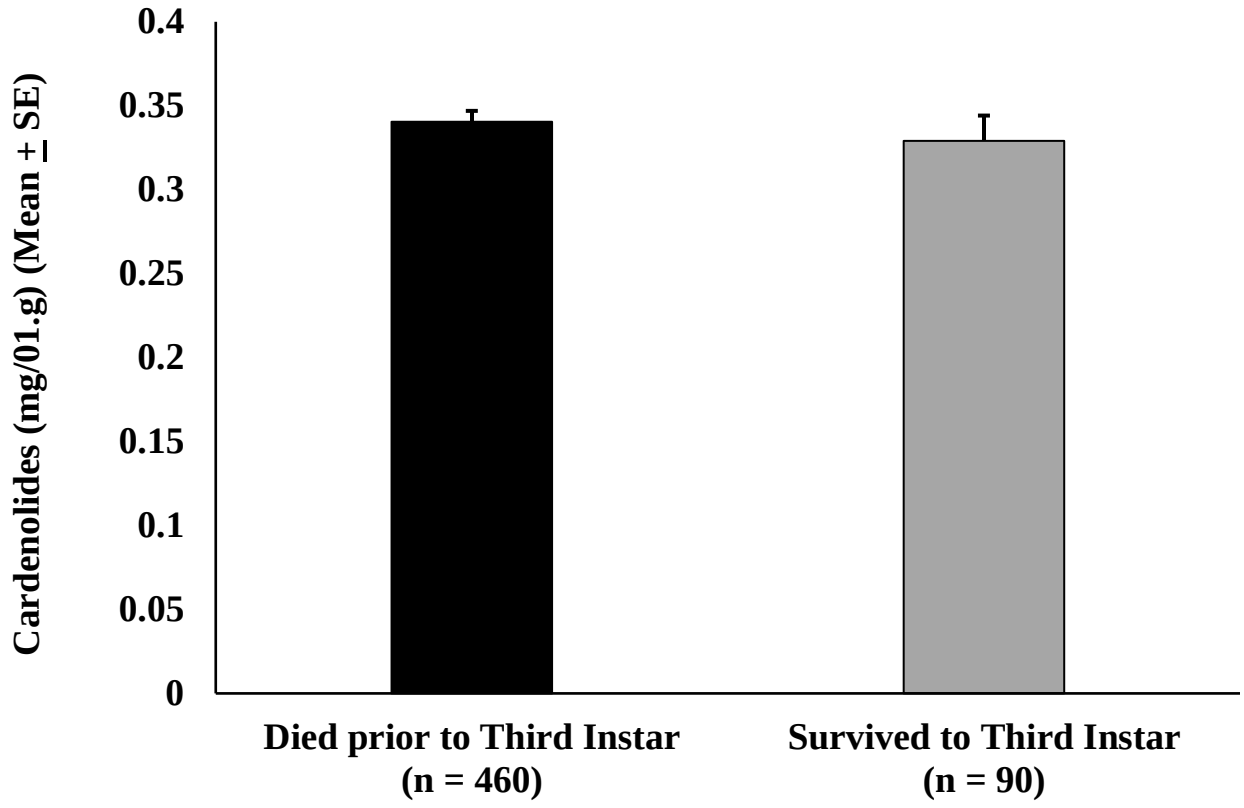

Figure 28. Mean cardenolide content of leaves from 460 host plants where larvae did not survive to the third instar and 90 host plants upon which larvae survived to the third instar. ANOVA, $F = 0.500$, $df = 1, 548$, $p = 0.4794$.

Table 47. Summary of the best fit model using stepwise multiple regressions of host plant cardenolides against physical attributes of the host plants. Full model statistics were: $F = 7.68$, $df = 7, 331$, $p < 0.0001$.

Parameter	DF	Estimate	Standard Error	t-value	Pr > t
Intercept	1	0.329947	0.012611	26.16	<.0001
Total Number of Ramets	1	-0.024396	0.006299	-3.87	0.0001
Total Number of Leaves	1	0.001405	0.000407	3.46	0.0006
Darkening of Leaf Blades	1	26.584001	7.956141	3.34	0.0009
Darkening of Leaf Veins	1	-0.034272	0.024135	-1.42	0.1565
Leaf Spot	1	12.920896	5.5325	2.34	0.0201
Shoot Tip Necrosis	1	-0.053459	0.014859	-3.6	0.0004
Browsed	1	0.339926	0.1041	3.27	0.0012

Only 12 of the 231 eggs reached the third instar, representing a crude survivorship of 5.2%. However, since most eggs were not found immediately after being laid, the data had to be corrected to account for losses that might have occurred prior to being found (Mayfield method, see spring survivorship results in section *b* above). Very few observations were made of females ovipositing, so the top 10% longest durations to hatching was used to estimate how long it takes an egg to hatch after being laid. This estimate was then used to develop Mayfield estimates of the survival rate of eggs. The percent survival of eggs was 40.9%, the percent survival of first instars was 33%, and the percent survival of second instars was 35.3%. These percentages were used to generate survivorship curves which were then compared to similar survivorship curves based on the spring data (Figure 29). The estimated survivorship to the third instar was 4.76%, considerably lower than the estimate of survivorship to the third instar for spring monarchs which was 15.5%. Assuming mortality rates are constant through the fifth instar, the extrapolated estimate of survivorship through the fifth instar is 1.0% for fall monarchs and 4.5% for spring monarchs.

Since Queen Butterflies and Monarch Butterflies coexist in the fall in north Texas, and since they utilize the same host plants, the data were examined for evidence that these two species are likely to compete. Two types of data were used for this analysis. First, daily observations were made of adult butterflies while looking for eggs. Second, the number of larvae of each species observed while searching for eggs was also counted. These data were then corrected according to the number of plants searched. Both types of counts were then tallied for each of five two-week time periods across the fall field season. Adult monarchs were at their peak abundance during the first portion of the study period, whereas adult queens gradually increased abundance throughout the season (Figure 30). Queens did not reach peak abundance until the end of September and beginning of October. Similarly, the number of monarch larvae observed per plant examined was highest near the middle of September whereas larval queens did not reach a peak until October (Figure 31). These data indicate that there is a temporal displacement between monarchs and queens in north Texas.

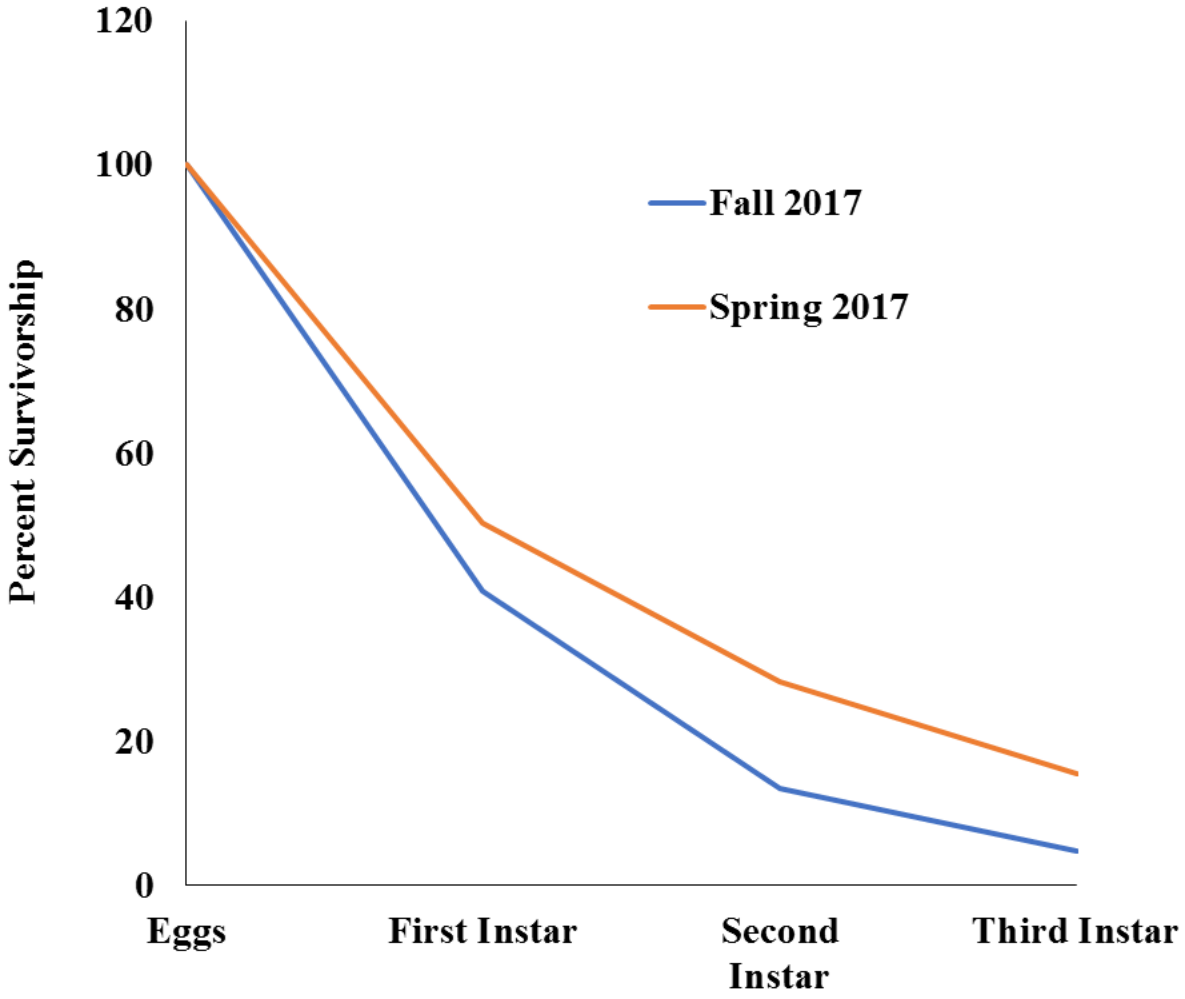

Figure 29. Survivorship curves of monarch eggs and larvae to the third instar in north Texas. The curves compare survivorship measured in spring 2017 with that measured in fall 2017. The fall study site was located 19 km south of the spring study site in Hopkins Co., Texas.

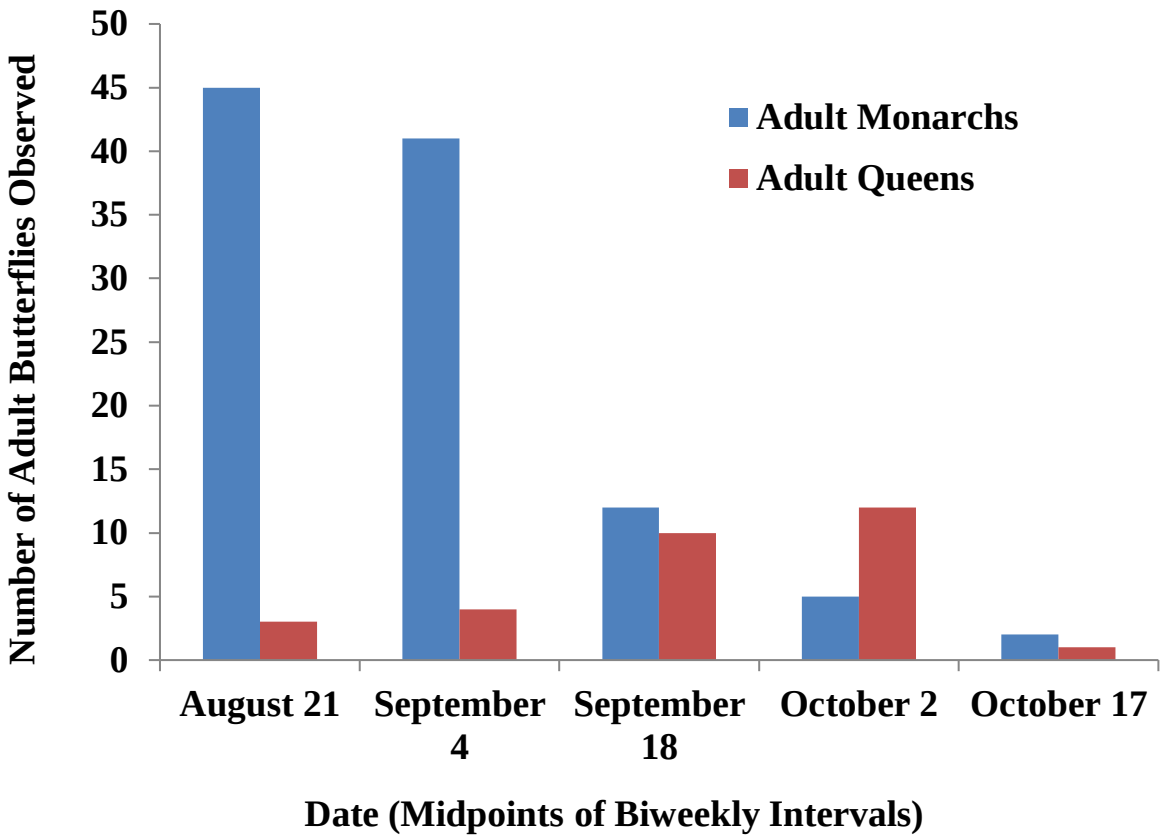

Figure 30. Occurrence of adult monarch and queen butterflies during the fall of 2017. The field season began on 15 August 2017 and ended 26 October 2017 and was divided into 5 two-week intervals. Dates on chart represent the midpoints of each two-week period.

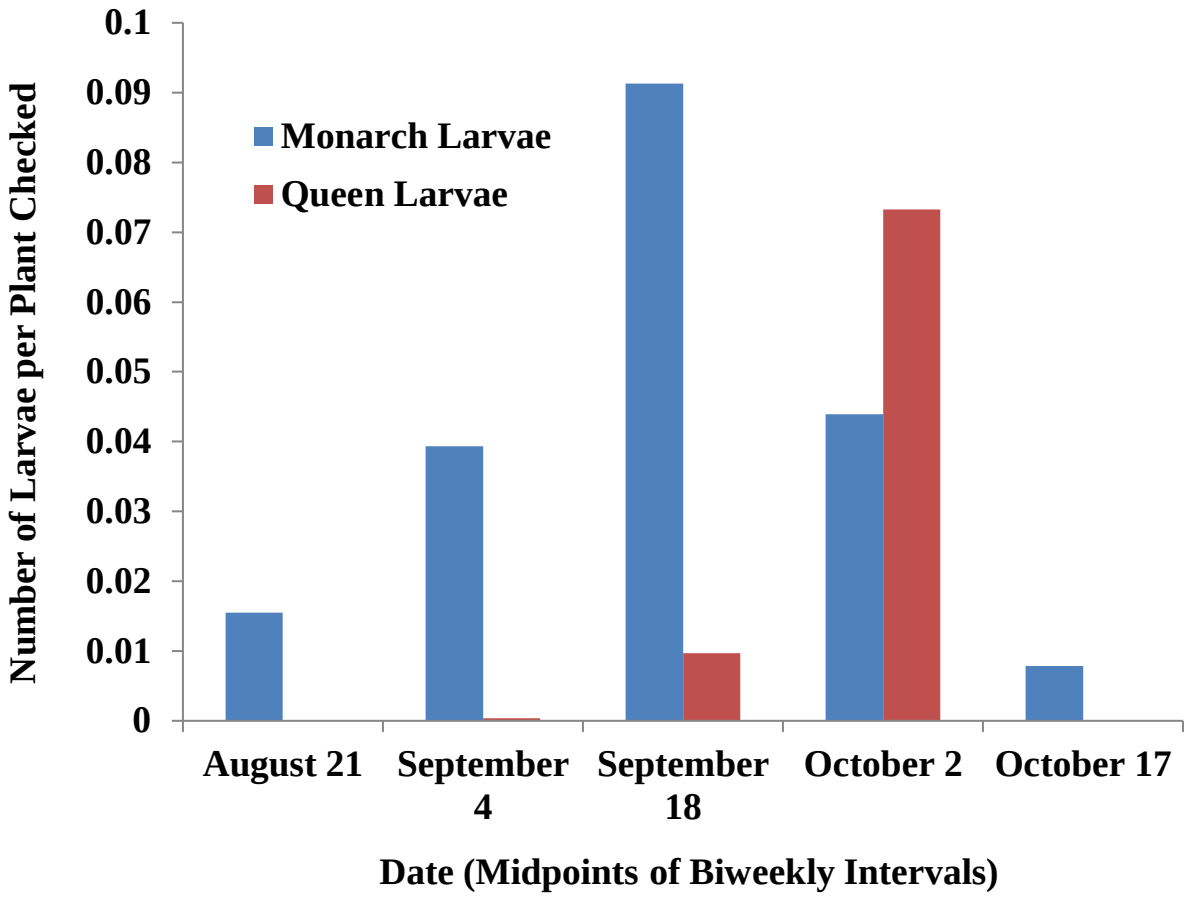

Figure 31. Occurrence of monarch and queen larvae during the fall of 2017. The field season began on 15 August 2017 and ended 26 October 2017 and was divided into 5 two-week intervals. Dates on chart represent the midpoints of each two-week period.

h. Monarch survival in north Texas compared among other studies

The survivorship data collected in spring and fall in north Texas was compared to survivorship studies conducted elsewhere using similar methodology (Figure 32). Data on spring survivorship collected in north Texas from 2016 through 2018 for control and RIFA suppressed treatments are higher all of the other studies with the exception of the study conducted in Florida. Survivorship was considerably higher than any other study in Texas. The lack of statistically significant difference between the control and the RIFA suppressed treatments indicate that controlling RIFA does not substantially increase monarch survivorship. Monarch survivorship in the fall of 2017 was lower than that recorded in the spring, but still higher than that recorded in Minnesota by DeAnda and Oberhauser (2015) and in Texas by Calvert (1996).

i. Synopsis of main results

There were differences between years in the phenology of events, the abundance of arthropods including RIFA and, to a lesser extent, differences in monarch egg and larval survival rates. The effect of suppressing of RIFA on monarch survival may vary between years. In some years RIFA suppression will have no effect on survival, in other years RIFA suppression results in a slight increase in monarch survival. This latter effect occurred in the cooler, drier year of 2018 when the overall abundance of arthropods and, in particular, the abundance of RIFA, was lowest. Monarch egg and larval survival seems to be most reduced when RIFA are artificially induced to occupy the plant as occurred in the RIFA enhanced treatments. Under normal (control) circumstances, RIFA are most likely to occupy a monarch host plant when there is a very high abundance of arthropods on that plant, particularly non-predatory species such as aphids, weevils, and leaf hoppers. However, under these circumstances, overall predator pressure is low and monarch survival is higher. Furthermore, monarch survival was highest when there were low numbers of RIFA on the plant. Survival was lower when there were many RIFA on the plant or when there were no RIFA on the plant.

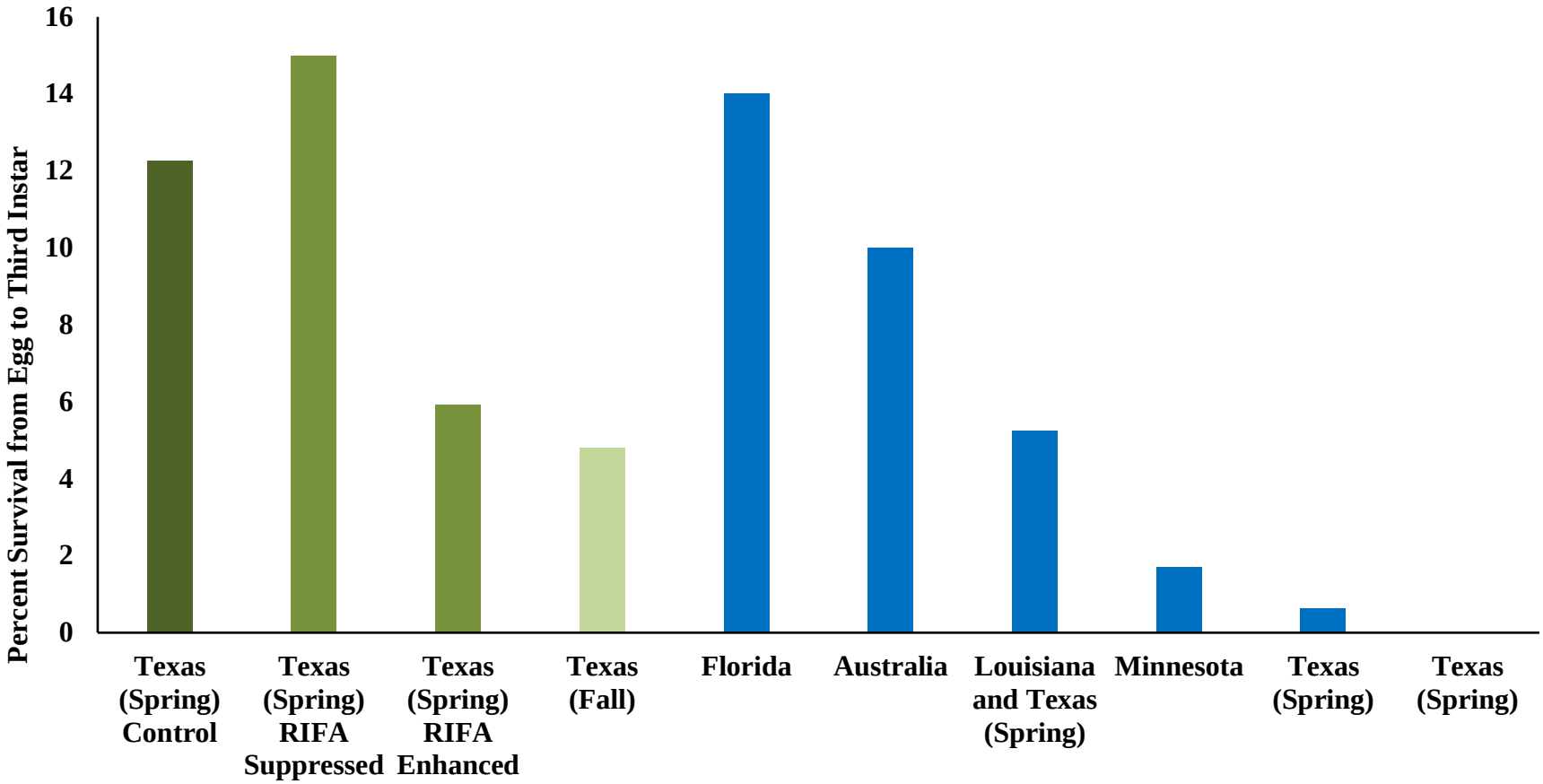

Figure 32. Survivorship data collected in spring and fall in north Texas in the current study (Green Bars) compared to other survivorship studies using similar methodology (Blue Bars). Sources: Florida, Cohen and Brower 1982; Australia, Zulucki and Kitching 1982; Louisiana and Texas, Lynch and Martin 1993; Minnesota, DeAnda and Oberhauser 2015; Texas, Calvert 1996; Texas, Calvert 2004 (control plants only).

Evaluation of the arthropod community on the host plants found that, though their role varied considerably, non-predatory arthropod populations were more important in predicting monarch survival than were predator populations. This was supported by both pairwise comparisons and stepwise logistic regression on control plants. When non-predatory arthropod populations were high, predator pressure was low. Monarch survival was higher on plants with lower predator pressure. On unmanipulated (control) host plants with low predator pressure, RIFA may have had a very slight negative impact on monarch survival. When predator pressure was high, other predators slightly influenced monarch survival, but RIFA had no detectable effect on monarch survival. At high predator pressure, increased numbers of alternate, non-predatory arthropods favored higher survivorship for monarch eggs and larvae.

The general tendency for non-predatory arthropods to exert greater influence on monarch survival than did predatory arthropods was consistent between years. However, the specific arthropods of importance varied and in some cases the direction of the influence of these groups changed from one year to the next. For 2017, there was no evidence that any predatory group directly influenced monarch survival. In 2018 when overall arthropod abundances were down, there was weak evidence that predators, other than ants and jumping spiders, might negatively affect monarch survival.

Experimental manipulation of RIFA densities affected the evenness and effective number of arthropod groups on the host plants. In general, arthropod groups were more abundant on host plants in the RIFA suppressed treatment and less abundant on host plants in the RIFA enhanced treatment. When these experimental host plants are included in the analyses, non-predatory arthropods become less important in predicting monarch survival and RIFA have a strong negative impact on monarch survival. However, it is important to note that the experimental enhancement caused RIFA to ascend onto host plants that they would otherwise not occupy. In the RIFA enhanced treatment 99.5% of plants had RIFA on them, whereas only about 30% of control plants were occupied by RIFA. The implication of this is that while it is possible to experimentally induce RIFA to occupy a host plant and increase monarch mortality, under normal circumstances RIFA are unlikely to ascend a host plant unless there are sufficiently large populations of favored arthropods on that plant. However, even when RIFA abundance

on the host plant is high and predator pressure due to RIFA is high, monarch survival is not negatively affected.

The terrestrial arthropods found in traps surrounding the host plants provide only an indirect indicator of effects on monarch survival because these data do not adequately include volant arthropods that are more likely to occur on the plants than in the traps. Consequently, the arthropod community occupying the plant and the terrestrial community adjacent to the plant are partly independent. The terrestrial arthropod populations surrounding host plants and their apparent impact on monarch survival varied markedly among years. In the year when the abundances of arthropods were lower, a greater number of arthropod groups affected monarch survival. Overall, a higher abundance of RIFA in the terrestrial arthropod community had a negative impact on monarch survival. However, this effect was small and was not evident when the data were analyzed separately for each year. Suppression of RIFA generally increased the abundances of other arthropods in the surrounding terrestrial community.

The average *A. viridis* host plant had just over two ramets, a total ramet length of over 60 cm, over 34 adult leaves and a cardenolide concentration of 0.338 mg/0.1g. This study was the first to document diseases associated with *A. viridis*. Leaf curling, herbivory, leaf miners, general necrosis, shoot tip necrosis, general wilt, browsing, and stem weevil damage were all attributable to arthropod or vertebrate influences, physical damage caused by trampling or wind, or water stress. The remaining characteristics, yellowing, leaf spotting, darkening of leaf veins, and darkening of leaf blades, were unrelated to soil parameters. Leaf spotting, darkening of leaf veins, and darkening of leaf blades were attributable to the presence of viruses, particularly Potyviruses. Yellowing had multiple causes including an association with aphids. Viral diseases may be vectored by milkweed herbivores, particularly milkweed bugs (Lygaeidae), weevils (Curculionidae), and, to a lesser extent, thrips (Thysanoptera).

The most common host plant characteristics that affected arthropods on the plant were the size of the host plant. Host plants with greater ramet length or more leaves, or both, had more arthropods. Pathological traits had remarkably little effect on the arthropods occupying the plant. The best predictor of monarch survival, based on host plant attributes, was a positive relationship between monarch survival

and the total number of leaves. Additionally, greater leaf curling, likely due to thermal or water stress, predicted lower monarch survival. None of the diseases or other pathologies had significant effects on monarch survival. Similarly, cardenolide concentrations were unrelated to monarch survival.

The survivorship of monarch eggs and larvae in the fall was considerably lower than that observed in spring. However, this survivorship is higher than estimates from Minnesota and the projected survivorship to the fifth instar is comparable to figures estimated for monarchs further north (3rd and 4th generation). There appears to be a temporal displacement between queen butterflies and monarchs such that competition between the two species in the fall is likely to be minimal. Consequently, monarch reproduction in Texas in the fall may be important for recruitment to overwintering sites in Mexico. Spring monarch survival among controls was much higher than that reported by other studies either for Texas or for locations outside of Texas.

Discussion

The survivorship of unmanipulated monarch eggs varied from about 10% to about 14% in the three years of this study, despite considerable variation in weather conditions. Only six other studies that use the same focal plant methodology provide data on survival to the third instar (Figure 32). These other studies vary considerably, from 0% survival in Texas (Calvert 1996) to 14% survival in Florida (Cohen and Brower 1982). The survival documented in the current study is high relative to that described in most of the other studies, indicating relatively high overall productivity of spring monarchs in north Texas.

Two other studies that followed focal individuals provided slightly different data that can be directly compared to the current study. In Wisconsin, survival from egg to first instar took about four days and was 13% (Prysby 2004). Another study, also in Wisconsin, found that survival of eggs to hatching was 30% (Borkin 1982). In the current study, the average four-day survival rate of control eggs was much higher at 72.9% and the survival of eggs to hatching was 58.6%. These data support the observation that spring monarch survival in north Texas is high relative to that observed in most other studies.

Several studies provide estimates of monarch survival from egg to fifth instar (Cohen and Brower 1982, Zalucki and Kitching 1982, Lynch and Martin 1993, Oberhauser et al. 2001 in Prysby and Oberhauser 2004, Prysby and Oberhauser 2004, and Nail et al. 2015). These estimates vary considerably, from 2% to 8% in Australia (Zalucki and Kitching 1982) to 10% to 20% in the upper mid-west of the U.S.A. (Prysby and Oberhauser 2004). For most of these studies, survival to the fifth instar varies from 4% to 13%. Some of this variation may be a product of differences in methodology (see discussion of citizen science data and potential sources of bias in Prysby and Oberhauser 2004).

Cohen and Brower (1982), working in Florida, found that larval mortality was constant for all age classes from hatching though the fifth instar. Based on this assumption, for the current study, monarch survival extrapolated to the fifth instar would vary from 2.2% in 2018 to 3.8% in 2017. These values fall into the low end of the estimates cited above. However, multiple studies have reported that larval monarch mortality is not constant across all age classes and that mortality is much higher for the youngest age classes (Zalucki and Kitching 1982, Lynch and Martin 1993, Oberhauser et al. 2001, Prysby and Oberhauser 2004, and Nail et al. 2015). In Australia, it was found that the mortality of eggs, first, second and third instars accounted for between 86% and 100% of the mortalities that occur prior to pupation (Zalucki and Kitching 1982). Similarly, in Louisiana and Texas, it was found that 97% of eggs failed to reach the third instar and that less than 10% of all mortalities occur after the third instar (Lynch and Martin 1993). If survivorship after the third instar is higher than prior to the third instar, then the estimates of survival to the fifth instar provided above for the current study (2.2% to 3.8%) are grossly underestimated. Consequently, without data on the survivorship of third through fifth instars, it is not possible to compare survivorship to the fifth instar in the current study with estimates from other studies.

Despite the uncertainty regarding survivorship to the fifth instar, the high survivorship observed in the current study implies that monarch productivity in the spring in north Texas is high. Interpreting the impact of this productivity on monarch populations and monarch declines is problematic because there is no baseline data on monarch productivity in Texas prior to the onset of monarch declines (i.e. prior to early 1990's, Thogmartin et al. 2017) or prior to the invasion of Texas by RIFA in the 1950's and

1960's (Cokendolpher and Phillips 1989). Without this data it is difficult to know precisely whether current spring productivity is sufficient or not. On the one hand, the conservative estimates of fifth instar survival for the current study are above replacement levels. However, if subsequent generations, which occupy a much larger geographic area, are to expand in numbers, then high productivity of the first generation may be extremely important.

In Texas, and elsewhere within their current distribution, RIFA have been implicated as affecting monarch egg and larval survival. A study conducted in Louisiana and east Texas implicated ants as important predators of monarchs, but did not identify the species of ant (Lynch and Martin 1993). They found that ants occurred on 42% of the host plants they monitored and observed ant depredation on monarch instars of all age classes. However, they found only a weak negative correlation between the presence of ants on the host plants and the presence of larval monarchs. In that study, the presence of ants on the host plants was primarily attributed to the flowering of the host plants (Lynch and Martin 1993).

Calvert (1996) observed 100% mortality of monarch eggs and larvae in a field in south-central Texas. In this study, 61 eggs were found along with three first instars. There was no evidence of any older instars at the site. Evidence of RIFA predation was limited to a single observation of RIFA depredating a first instar monarch and that 4% of host plants examined on the site held RIFA on them. However, the mound density at the site was 1011 mounds/ha. This is an extraordinarily high mound density, close to twice that of the current study, and much higher than the average mound density in North America which varies from 155 to 470 mounds/ha depending on whether the ants are monogyne or polygyne (Macom and Porter 1996, Porter et al. 1997). The extraordinarily high RIFA density at the study site used by Calvert (1996) may explain the high mortality observed at that site. However, it is also possible that the small sample size in that study may, in part, explain the lack of older instars observed.

In a follow-up study, Calvert (2004) used exclosures to minimize RIFA access to host plants. That study, based on over 700 eggs, found monarch survivorship to be 26 times higher inside the exclosures than outside the exclosures and RIFA densities were 3.4 times higher outside the exclosures than they were inside the exclosures. However, the study did not specifically isolate RIFA as the cause

of higher mortalities outside the exclosures because the effect of the exclosures on other predators and other arthropods was not measured. There are many other arthropods that prey on monarchs, including wasps, spiders, stink bug nymphs, syrphid fly larvae, ladybird beetles, assassin bugs, lacewings, and variety of other dipterans (De Anda and Oberhauser 2015, Oberhauser et al. 2015). Lastly, ants other than RIFA are important predators of monarch eggs and larvae (Prysby 2004) and the study by Calvert (2004) did not indicate whether predation rates were higher than would be expected from native ants.

In the current study the RIFA density varied from 528 mounds/ha in 2017 to 617 mounds/ha in 2018, considerably lower than the mound density reported by Calvert (1996) but substantially higher than average mound densities reported for the U.S.A (Macom and Porter 1996, Porter et al. 1997). However, in the current study, where survival of monarchs was high, 28.7% of plants associated with control eggs held RIFA, a percentage that is much higher than the 4% reported by Calvert (1996) and comparable to the 42% reported by Lynch and Martin (1993). In the current study, three measures of RIFA abundance, distance to nearest mound, number of mounds adjacent to host plants, and volume of mounds adjacent to the host plants, failed to show evidence for direct effects of RIFA abundance on monarch egg or larval survival. There was, however, a weak relationship between the number of RIFA captured in traps adjacent to the host plant and monarch mortality, but this trend occurred only when experimentally manipulated plants were included in the analysis.

A rather strong relationship was found between the number of RIFA on the host plant and the survival of monarch eggs and larvae. However, this relationship was not linear, such that eggs on host plants that held low numbers of RIFA had much higher survival than host plants with many RIFA and host plants that had no RIFA. Interestingly, the number of RIFA on a host plant was not correlated to the distance of the host plant to the nearest RIFA mound or the total number and total volume of RIFA mounds adjacent to the host plant. Rather, the number of RIFA on a host plant was more strongly predicted by the overall abundance of arthropods on the plant. This observation, that in some circumstances RIFA might enhance monarch survival, and that RIFA abundance on the plant is predicted

by the presence of other, mostly non-predatory arthropods on the host plant, suggests that indirect community level effects, are operating on the host plants (see arthropod community discussion below).

In this study, an attempt was made to measure the direct effects of RIFA on monarch survival by manipulating the density of RIFA on and adjacent to the host plants. These manipulations clearly showed that artificially drawing RIFA onto the host plants decreased the survivorship of monarch eggs and larvae. Gluing mealworms onto the host plant exacerbated monarch mortality by taking advantage of two aspects of ant foraging behavior. Since the mealworms, once consumed or removed from the plant, were replaced daily, the mealworms represented a relatively constant and predictable food resource. In this circumstance ants are most likely to create pheromone foraging trails to expedite exploitation of the resource (Mailleux et al. 2000). Secondly, many ant species employ local area searching in the vicinity of places where food has been found (Trainiello 1989). This latter behavior caused the ants to ascend further on to the host plant allowing the ants to opportunistically prey on the monarch eggs and larvae. Furthermore, monarch larvae are known to move up and down the host plant and to temporarily leave the host plant for a variety of reasons (Rawlins and Lederhouse 1981, Borkin 1982). A monarch larva traveling down the host plant stem to reach the ground would be forced to travel through the region of heavy ant activity, thereby putting that larva at high risk for predation by ants.

When chemical treatments that specifically targeted RIFA were used to reduce RIFA populations, the effect of this treatment on improving monarch survival was minimal despite the fact that RIFA numbers were almost completely eliminated from the treated area. In the two years of the study, RIFA suppression had no effect on survival in one year and only a slight positive effect on survival in the second year when arthropod populations were lowest. This result is somewhat surprising since a variety of studies have implicated ants as important factors in suppressing monarchs (Cohen and Brower 1982, Calvert 1996, Prysby 2004). RIFA have been implicated as affecting a broad variety of taxa (Wojcik et al. 2001, Holway et al. 2002). RIFA have been found to have negative impacts on some vertebrates (Kopachena et al. 2000, Allen et al. 2004) and negative community-wide impacts on arthropod populations (Porter and Savignano 1990, Morrison 2002, Epperson and Allen 2010). Porter and

Savignano (1990) reported that invasion of RIFA in central Texas reduced the species richness of terrestrial arthropods by 40%. In the current study, RIFA suppression caused the effective number of arthropod groups on host plants to be more than twice as high as the number of effective arthropod groups found on control plants. However, these differences did not appear to impact monarch survival, most likely because RIFA were one of 28 different types of predators found on host plants. There were many other predators available to compensate for the lack of RIFA predation on monarch eggs and larvae in the RIFA suppressed treatment.

In contrast to the above discussion, there is evidence that some arthropods benefit from the presence of RIFA (King and Tschinkel 2006) and, in some cases, there is a positive relationship between RIFA density and arthropod diversity (Morrison and Porter 2003). It may be relevant that many of these studies are more recent than those reporting negative impacts of RIFA. A common pattern among invading species is for the population to exhibit a population spike, followed by declining populations and, ultimately, a stabilized lower population (Williamson 1996, Simberloff and Gibbons 2004, Crooks 2005). RIFA first appeared in five counties in southeast Texas in 1953 and were present in the vicinity of the current study area in the late 1960's and early 1970's (Cokendolpher and Phillips 1989). RIFA have, therefore, been part of local ecosystems for over 40 years, providing sufficient time for them to adapt and stabilize relative to regional biotic and abiotic factors (Strayer et al. 2006). In central Texas, though initial surveys post-invasion by RIFA indicated severe declines in ant and arthropod diversity, the diversity and abundance of ants and arthropods had returned to pre-invasion levels twelve years later (Morrison and Porter 2003). Most likely, in north Texas 40 years post-invasion, RIFA have become integrated within local arthropod communities. Consequently, and given the diversity of predators found on monarch host plants, it is not surprising that the control of RIFA had little effect on monarch survivorship.

The arthropods that occupied *A.viridis* host plants represented a remarkably rich and dynamic community. There are several reasons why particular arthropods occupy milkweed plants. Some insects are milkweed specialists. Of the 16 species of insects known to specialize on milkweed plants (Betz et al.

1997), 10 were found to occur on *A.viridis* in the current study. During the time that monarch eggs and larvae occupy milkweed plants in north Texas, the plants expend considerable energy blooming. Milkweed flowers attract a wide diversity of arthropods. For example, *A. tuberosa* flowers in Arizona are visited by over 80 different species of arthropods (Fishbein and Venable 1996) and *A. viridis* flowers in Oklahoma are visited by over 23 families of insects (Liaw 2017). In addition, the stout growth form of *A.viridis* plants make them attractive to insects that seek physical structures on which to rest or form harborages. For example, many spiders select plants based on their architecture (Vasconellos-Neto et al. 2017) and this seemed to be particularly true of the jumping spiders observed in the current study. Many arthropods are simply transient, using the milkweed plant as a temporary resting place. All of these arthropods, in turn, attract a large variety of predators to the host plants (De Anda and Oberhauser 2015, Oberhauser et al. 2015). In the current study, 28 different types of arthropod predators were found on monarch host plants. These predators represented four of the five most abundant arthropods on the host plants and the top two most frequent arthropods.

In the context of the numerous arthropods on the host plants, it seems unlikely that predators arrived on the host plant specifically seeking the eggs and larvae of monarchs. Monarch eggs and young instars are too small and too few in number to be a specific target for any particular predator. Consumption of these eggs and larvae are, therefore, opportunistic in nature.

The arthropod occupancy of host plants varied considerably and these variations had implications on the survival of monarch eggs and larvae. Importantly, in the combined data for control plants, none of the analyses identified RIFA as influencing monarch survival. Only predators other than ants and jumping spiders had any negative effect on monarch survival and this effect was slight at best. In general, it was increased numbers of non-predatory arthropods that improved the survivorship of monarch eggs and larvae. Furthermore, there were density dependent effects regarding the proportion of predators on the host plants relative to the number of non-predatory arthropods on the plant. As the number of non-predatory arthropods increased, predator pressure generally decreased, and monarch survival was highest at low predator pressure. However, the positive influence of the number of non-predatory arthropods on

monarch survival was most evident on host plants with high predator pressure. Furthermore, when high predator densities and high predator pressure occur on the same plant, monarch survival is not decreased. These findings suggest that rather complex indirect community level effects are occurring on these plants.

The overall complexity of the arthropod community on the host plants makes it difficult to isolate single causal agents leading to monarch mortality or survival. The variety and intensity of the numerous ecological interactions is, for the most part, beyond the scope of this project. However, several aspects of the interactions among potential prey species and predators are important. In ecological communities, indirect effects occur when the impact of a species or group of species (RIFA in this study) on another species or group of species (monarchs in this study) is altered by the presence of a third species or group of species (other arthropods in this study) (Wootton 1994, Mittelbach 2012). Indirect effects are important for promoting species richness among trophic levels. For example, top-down regulation by predators has been shown to increase herbivore diversity (Amundrud et al. 2015) and in some cases preferential predation by a predator on one prey species can lead to increases in the population of less preferred prey species (Frago and Godfray 2014, Prado and Frank 2014). In the current study, higher abundances of other arthropods relative to predator abundances favored improved monarch survival. This may be due to an indirect effect in which predators, such as spiders and ants, preferentially fed on other phytophagous insects inhabiting the milkweed plants and, as a result, lowered predator pressure and, consequently, improved monarch survival. Optimal foraging theory demonstrates unequivocally that predators ignore less profitable prey when more profitable prey are available and that even slight differences in profitability can cause a prey species to be eliminated from the diet of a predator (Giraldeau 2008, Prado and Frank 2014).

There is reason to expect other species of insects on milkweed plants to be preferable to predators because not all phytophagous insects on milkweed plants sequester cardenolides or are as efficient as monarchs in sequestering these compounds (Isman et al. 1977) and at least some polyphagous invertebrate predators show an aversion to prey with high levels of cardenolides (Raynor 2004). It may be significant that weevils, a milkweed specialist that does not sequester cardenolides (Fordyce and

Malcolm 2000), were important arthropods associated with reduced monarch mortality on host plants when predator pressure was high. Similarly, all of the other arthropods whose increased abundance was associated with increased survivorship do not sequester cardenolides. All of these species are likely to present more profitable alternative prey for predators occupying monarch host plants.

There is scant detail available about the foraging preferences of the arthropod predators observed on the host plants in this study. However, the nutritional content of prey can have important effects on prey choice even in generalist arthropod predators (Schmidt et al. 2012). RIFA are well known as generalist predators, being attracted to lipids and proteins (Ricks and Vinson 1970, Stein et al. 1990). However, they are also strongly attracted to nectar and other sugar sources (Stein et al. 1990, Lanza et al. 1993, Vander Meer et al. 1995) and, in many contexts, collect more liquid food in the form of carbohydrates than solid food (Stein et al. 1990, Tennant and Porter 1991). The extent to which RIFA seek carbohydrates or proteins depends on the current nutritional status of the colony (Cassill and Tschinkel 1999). During cooler periods, RIFA seek carbohydrates and during warmer periods they favor proteins (Stein et al. 1990). During wet weather RIFA forage for more carbohydrates (Ali and Reagan 1986). Carbohydrate intake also varies with habitat (Vogt et al. 2002). RIFA workers that have been food deprived prefer carbohydrates over amino acids (Cassill and Tschinkel 1999) suggesting that when resources are scarce, carbohydrates might be preferred to maintain worker survival. When demand for carbohydrates is strong, workers foraging for carbohydrates continue to do so to the exclusion of protein sources. Similarly, when demand for protein is strong, workers foraging for proteins do so to the exclusion of carbohydrate sources. Changes in worker foraging preferences only occur when the nutritional needs of the workers change (Cassill and Tschinkel 1999). Thus, the diet choices of RIFA are affected by the nutritional needs of the colony and, since arthropods vary considerably in nutritional content (Wilder and Eubanks 2010), RIFA prey choice among available arthropod types will vary accordingly.

In the current study, under natural, control conditions, RIFA ascended onto a monarch host plant in the largest numbers when there was a large population of other arthropods on the plant, specifically

aphids, weevils, and leaf-hoppers. RIFA are known to tend ants for honeydew (Stein et al. 1990, Wojcik et al. 2001) and were observed to do so in this study. Because leaf-hoppers are phloem feeders and because the weevils in this study are both phloem feeders and nectarivores, these species are likely to also be high in carbohydrates. Consequently, the RIFA on these control plants were likely foraging for high-carbohydrate foods. In this context, if monarch eggs and larvae contain fewer carbohydrates, they would not be preferred as a food source and would incur some level of security against RIFA predation. On the other hand, on the RIFA enhanced plants, the presence of a reliable source of high lipid and high protein food (mealworms, Ng et al. 2002) would attract RIFA workers seeking proteins and lipids. These workers would be more likely to prey on monarch eggs and larvae and this would result in the much higher rate of mortality observed in the RIFA enhanced treatment.

In addition to potentially exercising prey selectivity among herbivorous prey types, RIFA, as generalist predators, frequently engage in intraguild predation (Eubanks et al. 2002). Intraguild predation has been demonstrated to have important effects on the strength and structure of arthropod community interactions (Polis et al. 1989, Vance-Chalcraft et al. 2007, Gagnon et al. 2011), particularly as it affects herbivore populations (Rosenheim et al. 1993, Bucher et al. 2015, Hagler and Blackmer 2015) Consequently, if RIFA consume other predators on the host plants, they will disrupt the type of predator pressure exerted on monarch eggs and larvae. This would be particularly true if RIFA depredation selectively affects different kinds of predators. For example, in Alabama cotton fields, it was found that RIFA reduced the survival of lady beetles and lacewing larvae, but had no effect on the survival of spiders (Eubanks et al. 2002). Intraguild predation, along with a failure to specifically search for monarch eggs and larvae, might explain why monarch survival was higher on plants that held a small number of RIFA than it was on plants that had no RIFA. It would also explain why monarch survival was high on plants that had both high numbers of RIFA and high predator pressure.

There are precedents for indirect effects in monarch associated predator-prey systems. In both field and laboratory studies of predation on monarch larvae by ladybugs (*Harmonia axyridis*), monarch larvae had increased survival when there were higher densities of an alternate prey in the form of aphids

(*Aphis nerii*) (Koch et al. 2005) (but see Prysby 2004 for contrast). There is also precedence for the idea that RIFA may reduce predation on some phytophagus species in the presence of more profitable prey. In cotton fields, RIFA release cotton aphid (*Aphis gossypii*) from predation by ladybug larvae (Coleoptera: Coccinellidae) and lacewing larvae (Neuroptera: Chrysopidae) (Kaplan and Eubanks 2002). These types of indirect effects explain why, in the context of the arthropod community on the host plants in the current study, the survival of monarch eggs and larvae was more closely tied to the type and abundance of non-predatory arthropods than it was to the presence of predators.

Evaluation of terrestrial arthropods around host plants in the control and RIFA suppressed treatments did not yield strong predictive models of monarch survival. This is probably because the terrestrial arthropods only represent a subset of the overall arthropod community affecting monarch eggs and larvae. RIFA were detected as having a slight, albeit not statistically significant, negative impact on monarch survival in the combined data. However, when only control plants were evaluated, or if the data were stratified by year, this effect was no longer evident. These results suggest that while RIFA are predators on monarchs, and removing them removes their effect on monarchs, in natural circumstances, the effect of RIFA is for the most part ameliorated by indirect effects of other arthropods and by volant species not represented in the terrestrial arthropods captured in the traps. However, the observation that, in 2018, when the overall abundances of arthropods was low, more groups of arthropods affected monarch survival, is consistent with the preceding discussion that indirect effects operating within the community are also subject to density dependent effects. It highlights the importance that overall species diversity might have on the ability of a community to buffer predation in the event of population fluctuations and the impact of those fluctuations on monarch survivorship.

In this study, plant characteristics varied considerably in growth form and in the presence of pathologies. Some of these pathologies were due to abiotic factors (leaf curling, general necrosis, general wilt) whereas others were due to arthropods and vertebrates (leaf miners, herbivory, shoot-tip necrosis, browsing, and stem-weevil damage). Three other pathologies, leaf spotting, darkening of leaf veins, and darkening of leaf blades, were attributable to the presence of viruses, particularly Potyviruses. This study

is the first to describe and identify these viral diseases in *A. viridis*. The presence of viral diseases was associated with, and possibly vectored by, milkweed bugs, weevils, and thrips. However, none of these insects are known to vector the viruses detected (Nault 1997). It is interesting that aphids were not associated with any of these viral diseases because aphids and to a lesser extent mites and whiteflies are the only documented vectors of Potyviruses (Nault 1997). However, very little is known about viral transmission in milkweed plants (Wiley 2009) and the association between arthropods and found in this study requires more detailed investigation.

Despite the fact that many plants appeared to suffer rather severe pathological symptoms, these symptoms generally had remarkably little effect on the arthropods occupying the plants or on the survival of monarch eggs and larvae. The only characteristics of host plants that increased the survival of monarch eggs and larvae as well as the abundance of other arthropods was the size of the plant. Larger plants were more likely to support more arthropods and favored higher survival of monarchs. This is a common phenomenon that has been observed in a wide variety of herbivorous arthropods including monarchs (Price 1991, Agrawal 2005).

The cardenolide content of *A viridis* host plants on the study site was well within the range reported for this species in Louisiana and Florida (Lynch and Martin 1987, Malcolm and Brower 1989). However there appeared to be no effect of cardenolide content and arthropod abundance or monarch survival. Other studies have found that monarch herbivory induces higher production of host plant cardenolides (Malcolm 1994, Malcolm and Zalucki 1996, Rasmann and Agrawal 2011) which, in turn, may lead to mortality especially in young instars (Malcolm and Zalucki 1996). However, in the current study there was no evidence of cardenolide induction in *A. viridis* in response to monarch herbivory. Furthermore, unlike previous findings (Zalucki et al. 1990) there was no evidence that monarch females selected host plants based on cardenolide content.

This study only followed eggs and larvae to the third instar and, consequently, could not quantify the effects of plant pathology on older instars. Furthermore, the effects of plant pathology on monarchs may be subtler, expressed as differential growth rates or lower larval mass as has been documented in

other studies (e.g. Zalucki and Brower 1992, Zalucki et al., 2001, Lavoie and Oberhauser 2004, Agrawal 2005, Pocius et al. 2017). Since growth rates were not measured in the current study, these effects remain to be investigated.

Fall reproduction of monarchs in Texas was first quantified by Calvert (1999) and later by Prysby and Oberhauser (2004). The origin of these migrants appears to be primarily from early northern migrants that either fail to enter diapause (Calvert 1999) or which break diapause as they migrate south (Borland et al. 2004, Batalden and Oberhauser 2015). Fall breeding individuals collected in Texas had isotopic signatures confirming that most of these butterflies originated in the northern U.S.A. and southern Canada with a very few individuals originating in the southern plains (Flockhart et al. 2013). It is believed that the stimulus to break diapause in the south is the presence of viable milkweed plants (Batalden and Oberhauser 2015). The butterflies that result from the eggs laid in the fall in Texas appear to be in diapause and, therefore, could contribute significantly to the overwintering population in Mexico (Batalden and Oberhauser 2015) but this aspect needs more detailed study. Nonetheless, stable isotope analyses of butterflies in winter roosts indicate that, on average, about 11% of the winter roost population originates in the southwestern portion of the eastern monarchs breeding distribution and in some years this contribution is as high as 25% (Flockhart et al. 2017). Consequently, fall reproduction in Texas could represent an important component of the overwintering population in Mexico.

The current study is the first to document the survival of fall monarchs. There was little evidence that monarchs and queens experienced competitive effects in the fall in north Texas. It was found that the survivorship of fall monarchs was considerably lower than it was in spring. However, the level of survivorship in fall in Texas was comparable to survivorship recorded in the northern U.S.A. where the other migrants heading to Mexico originate. This means that fall monarch production can be a very important component in the overwintering populations in Mexico. Furthermore, the year that the current study was conducted was a particularly dry year. Fall monarch production in normal or wetter years might be considerably higher, making this generation even more important in helping to buffer population fluctuations inherent in the more northern generations (Inamine et al. 2016).

Recommendations for Management of Monarch Populations in Texas

This study found that monarch survival in spring in north Texas is high and controlling RIFA may not be an effective means of improving monarch success. In some years chemical control of RIFA would be contraindicated. Furthermore, this study found that monarch success is highest in a diverse arthropod community that includes some RIFA. Based on these findings the following management recommendations are made:

1. Control of RIFA is unnecessary in most circumstances. In the Calvert 1996 study the mound density was 1011 mounds/ha. In situations such as this, with extraordinarily high mound densities, chemical control might be useful. However, the mound density in the current study was as high as 617 mounds/ha and did not find RIFA control to be an effective means of improving monarch survival. Since the average mound density in the U.S. varies from 155 to 470 mounds/ha (Macom and Porter 1996, Porter et al. 1997) it is likely the most sites in Texas will not require active, direct control of RIFA abundance.
2. Monarch survival is enhanced in ecological communities that contain a diverse array of arthropods with multiple trophic interactions. Such a community is most likely to occur through the encouragement of a high diversity of forbs and grasses (Hertzog et al. 2016, Welti et al. 2017). Management strategies that increase milkweed abundance while simultaneously increasing forb and grass diversity should be employed. Such management plants should include appropriately timed mowing and burning and seeding if necessary.
3. Monarch reproduction in the fall in Texas may be an important contribution to overwintering monarchs in Texas. Management for fall monarchs should be similar to those for spring monarchs and should encourage native milkweed regeneration and high plant diversity through appropriately timed mowing, burning, and seeding if necessary.

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Appendix 1. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Hemiptera, Aphidoidea	Aphid	281	31	24445	222	24726	253
Hymenoptera, Formicidae, *Solenopsis invicta*	Red Imported Fire Ant	3668	164	7046	154	10714	318
Hymenoptera, Formicidae, *Monomorium minimum*	Little Black Ant	2098	153	1303	82	3401	235
Hymenoptera, Formicidae, others	Other Ants	182	52	1209	48	1391	100
Araneae, Salticidae	Jumping Spider	590	211	353	177	943	388
Arachnida, Acari, Mites	Mite	234	116	600	80	834	196
Coleoptera, Curculionidae, Baridinae	Flower Weevil	372	64	343	51	715	115
Hemiptera, Lygaeidae, *Oncopeltus fasciatus*	Large Milkweed Bug	542	134	82	37	624	171
Diptera, unknown	Other Fly	306	160	290	109	596	269
Hemiptera, Cicadomorpha	Leafhopper	236	139	306	143	542	282
Coleoptera, Curculionidae, Molytinae	Stem Weevil	432	88	75	32	507	120
Coleoptera, Dermestidae	Dermestid Beetle	443	68	20	19	463	87
Coleoptera, Chrysomelidae, Alticini	Flea Beetle	387	148	21	12	408	160

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Aranea, Unknown	Other Spider	269	126	70	54	339	180
Orthoptera, Caelifera	Grasshopper	185	101	122	66	307	167
Coleoptera, Chrysomelidae	Other Leaf Beetle	72	57	105	48	177	105
Thysanoptera	Thrip	73	36	78	45	151	81
Coleoptera, Unknown	Other Beetles	138	57	11	9	149	66
Hemiptera, Heteroptera	Other True Bugs	105	64	35	29	140	93
Hymenoptera, Apocrita, unknown wasps	Wasp	79	57	43	43	122	100
Coleoptera, Curculionidae, Entiminae	Broad-nosed Weevil	94	44	11	7	105	51
Araneae, Araneidae	Orb-weaver Spider	34	23	69	48	103	71
Othoptera, Tettigoniidae	Katydid	33	29	61	28	94	57
Araneae, Thomisidae, *Misumena vatia*	Goldenrod Crab Spider	51	29	37	25	88	54
Diptera, Chironomidae	Midge Fly	46	45	39	34	85	79

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Araneae, Thomisidae	Other Crab Spider	34	28	34	24	68	52
Araneae, Oxyopidae	Lynx Spider	47	39	18	17	65	56
Coleoptera, Coccinellidae, *Harmonia axyridis*	Asian Ladybeetle	28	22	21	11	49	33
Hemiptera, Lygaeidae, *Lygaeus kalmii*	Small Milkweed Bug	30	23	18	16	48	39
Araneae, (Lycosidae, Agelenidae, Pisuridae)	Wolf, Grass, and Nursery-web Spiders	34	31	13	13	47	44
Araneae, Tetragnathidae	Long-jawed Orb Weaver Spider	35	32	8	5	43	37
Hemiptera, Lygaeidae, unknown	Other Seed Bug	20	15	20	7	40	22
Coleoptera, Coccinellidae, *Coccinella septempunctata*	Seven-spotted Ladybeetle	21	18	19	17	40	35
Arachnida, Opiliones	Harvestman	6	6	33	13	39	19
Coleoptera, Cerambycidae	Longhorn Beetle	26	16	10	5	36	21

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Coleoptera, Scarabaeidae	Scarab Beetle	25	17	8	8	33	25
Hymenoptera, Apidae, Xylocopa sp.	Carpenter Bee	27	19	5	5	32	24
Coleoptera, Coccinellidae, Larva	Ladybeetle Larva	2	2	28	8	30	10
Diptera, Syrphidae, larvae	Flower Fly, larvae	0	0	27	13	27	13
Myriapoda, Diplopoda	Millipede	22	15	4	4	26	19
Diptera, Calyptratae	Other Calyptrate Fly	0	0	25	21	25	21
Phasmatodea	Stick Insect	13	13	11	11	24	24
Hemiptera, Miridae	Plant Bug	7	6	17	9	24	15
Lepidoptera, larva	Caterpillar	11	11	12	12	23	23
Hymenoptera, Apidae, Apis sp.	Honey Bee	19	10	3	3	22	13
Hymenoptera, Anthophila, Unknown	Other Bee	16	10	6	6	22	16
Insecta, Unknown egg	Insect Egg	20	2	0	0	20	2
Hemiptera, Reduviidae	Assassin Bug	8	6	10	9	18	15

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Diptera, Muscidae	House Fly	8	8	9	9	17	17
Hemiptera, Pentatomoidea	Stink bug, non-predatory	12	7	4	4	16	11
Collembola	Springtail	16	12	0	0	16	12
Coleoptera, Cantharidae	Soldier Beetle	11	9	5	4	16	13
Diptera, Tachinidae	Tachinid Fly	9	9	5	5	14	14
Arachnida, Acari	Tick	6	4	8	5	14	9
Diptera, Syrphidae, adult	Flower Fly, adult	9	9	4	4	13	13
Diptera, Sarcophagidae	Flesh Fly	3	3	9	9	12	12
Mollusca	Snails and Slugs	12	10	0	0	12	10
Neuroptera, adult	Lacewing	1	1	10	9	11	10
Lepidoptera, Heterocera	Moth	7	4	4	3	11	7
Hemiptera, Pseudococcidae	Mealybug	0	0	11	4	11	4
Coleoptera, Elateridae	Click Beetle	7	5	3	3	10	8
Trichoptera	Caddisfly	7	7	3	3	10	10

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Othoptera, Grylidae	Field Cricket	5	4	3	3	8	7
Diptera, Culicidae	Mosquito	1	1	7	7	8	8
Diptera, Tipulidae	Cranefly	0	0	8	7	8	7
Lepidoptera, Papilionoidea	Butterflies and Skippers	7	7	0	0	7	7
Neuroptera, larva	Lacewing Larva	0	0	7	5	7	5
Hymenoptera, Apidae, Bombus sp.	Bumblebee	6	6	1	1	7	7
Hemiptera, Coreidae	Leaf-footed Bug	3	2	4	2	7	4
Araneae, Philodromidae	Running Crab Spider	0	0	7	7	7	7
Isopoda	Isopod	6	5	0	0	6	5
Coleoptera, Staphylinidae	Rove Beetle	1	1	5	5	6	6
Diptera, Calyptratae, unknown	Calyptrate Fly	2	2	4	4	6	6
Araneae, Salticidae, *Myrmarachne* sp.	Ant-mimic Jumping Spider	2	2	4	3	6	5

Appendix 1 Continued. Raw counts and frequencies of arthropods observed on Asclepias viridis plants while monitoring 816 individuals on 529 plants in northeast Texas. Predatory species highlighted in yellow, milkweed herbivores are highlighted in green.

		2017		2018		Both Years	
Scientific Name	Common Name	Count	Frequency	Count	Frequency	Count	Frequency
Hemiptera, Pentatomidae, Asopinae	Predatory Stink Bug	2	2	3	2	5	4
Coleoptera, Carabidae	Ground Beetle	4	4	1	1	5	5
Diptera, Drosophilidae	Fruit Fly	5	2	0	0	5	2
Blattodea, Isoptera	Termite	0	0	5	5	5	5
Coleoptera, Tenebrionidae	Darkling Beetle	2	2	2	2	4	4
Hymenoptera, Vespidae	Vespid Wasp	3	3	1	1	4	4
Ephemeroptera	Mayfly	2	2	0	0	2	2
Hymenoptera, Halictidae	Sweat Bee	0	0	2	2	2	2
Mecoptera	Scorpion Fly	1	1	0	0	1	1
Coleoptera, Curculionoidea, Unknown	Other Weevil	1	1	0	0	1	1
Lepidoptera, *Danaus gilippus*, adult	Queen Butterfly	1	1	0	0	1	1
Coleoptera, Coccinellidae, *Cryptolaemus sp.*	Mealybug Destroyer	0	0	1	1	1	1

Appendix 2. Definitions, raw counts, and frequency of occurrence for host plant arthropod groups used in statistical analyses. Raw counts and frequencies are number of individuals or occurrences associated with 816 monarch eggs on 529 host plants.

		2017		2018		Both Years	
Arthropod Group	Included Taxa	Raw Count	Raw Frequency	Raw Count	Raw Frequency	Raw Count	Raw Frequency
Hemiptera, Aphidoidea	Aphids	281	31	24445	222	24726	253
RIFA, Formicidae, *Solenopsis invicta*	RIFA	3668	164	7046	154	10714	318
Formicidae, *Monomorium minimum*	Little Black Ants	2098	153	1303	82	3401	235
Formicidae	Other Ants	182	52	1209	48	1391	100
Curculionidae	Coleoptera: All Weevils (Curculionidae)	899	130	429	72	1328	202

Appendix 2 continued. Definitions, raw counts, and frequency of occurrence for host plant arthropod groups used in statistical analyses. Raw counts and frequencies are number of individuals or occurrences associated with 816 monarch eggs on 529 host plants.

		2017		2018		Both Years	
		Raw Count	Raw Frequency	Raw Count	Raw Frequency	Raw Count	Raw Frequency
All Other Arthropods	Stick Insects (Phasmatodea), Crickets (Gryllidae), Click Beetles (Elateridae), Darkling Beetles (Tenebrionidae), Leaf-footed Bugs (Coreidae), Seed Bugs (Lygaeidae), Plant Bugs (Miridae), Shield Bugs (Pentatomoidea, non-predatory), unidentified Wasps (Apocrita), Millipedes (Diplopoda), Springtails (Collembola), Ticks (Acari), Butterflies, Skippers, Moths (Lepidoptera), Slugs and Snails (Mollusca), Caddisflies (Trichoptera), Mayflies (Ephemeroptera), Harvestmen (Opiliones), Bees (Hymenoptera, Apidae), Grasshoppers (Caelifera), Katydids (Tettigoniidae), Unidentified True Bugs (Hemiptera, Heteroptera), Flesh Flies (Sarcophagidae), Tachinid Flies (Tachinidae), House Flies (Muscidae), and unknown Calyptrate flies	730	222	461	192	1191	414
Other Predators (not including ants and jumping spiders	Rove Beetles (Staphylinidae), Soldier Beetles (Cantharidae), Ground Beetles (Carabidae), Assassin Bugs (Reduviidae), Predatory Stink Bugs (Pentatomidae, Asopinae), Vespid Wasps (Vespidae), Scorpionflies (Mecoptera), Lacewings (Neuroptera), and Hoverflies (Syrphidae), Ladybeetles (Coleoptera, Coccinellidae), Wolf spiders (Lycosidae), Grass Spiders (Agelenidae), Nursery Web Spiders (Pisuridae), Long-jawed Orb Weavers (Tetragnathidae), Lynx Spiders (Oxyopidae), Crab Spiders (Thomisidae), and unidentified spiders	601	227	431	190	1032	417

Appendix 2 continued. Definitions, raw counts, and frequency of occurrence for host plant arthropod groups used in statistical analyses. Raw counts and frequencies are number of individuals or occurrences associated with 816 monarch eggs on 529 host plants.

		2017		2018		Both Years	
		Raw Count	Raw Frequency	Raw Count	Raw Frequency	Raw Count	Raw Frequency
Araneae, Salticidae	All Jumping Spiders (Salticidae)	592	212	357	180	949	392
Acari	Mites (Arachnida, Acari)	234	116	600	80	834	196
Diptera < 5 mm	Midge Flies (Chironomidae), Fruit Flies (Drosophilidae), Mosquitoes (Culicidae), and unknown flies	330	169	331	131	661	300
Lygaeidae, *Oncopeltus fasciatus*	Large Milkweed Bugs	542	134	82	37	624	171
Chrysomelidae	Coleoptera: Flea beetles (Chysomelidae, Alticini) and all other leaf beetles (Chysomelidae)	459	173	126	54	585	227
Auchenorrhyncha	Leafhoppers (Hemiptera,Auchenorrhyncha)	236	139	306	143	542	282
Dermestidae	All Dermestid Beetles (Coleoptera, Dermestidae)	443	68	20	19	463	87

Appendix 2 continued. Definitions, raw counts, and frequency of occurrence for host plant arthropod groups used in statistical analyses. Raw counts and frequencies are number of individuals or occurrences associated with 816 monarch eggs on 529 host plants.

		2017		2018		Both Years	
		Raw Count	Raw Frequency	Raw Count	Raw Frequency	Raw Count	Raw Frequency
Other Milkweed Herbivores	Aphids (Aphididae), Small Milkweed Bugs (*Lygaeus kalmia*), Milkweed Longhorn Beetles (*Tetraopes texanus*), and Thrips (Thysanoptera)	130	67	106	65	236	132
Coleoptera Unidentified	Unidentified Beetles (Coleoptera)	138	57	11	9	149	66

Appendix 3. Arthropod taxa captured in sticky traps adjacent to *Asclepias viridis* monarch host plants. Sample sizes in parentheses are the number of monarch eggs associated with each taxon. Data sorted by number captured in both years. Taxa highlighted in red are predators.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=638)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Red Imported Fire Ant (Solenopsis invicta)	9551	84.13	3456	75.07	13007	78.93
Unknown Fly (Diptera)	4447	97.42	2209	91.23	6656	93.87
Isopod (Crustacea – Isopoda)	783	49.45	4771	71.51	5554	62.11
Aphid (Hemiptera – Aphoidea)	1725	88.93	3179	92.88	4904	91.19
Mite (Arachnida – Acari)	1424	85.98	1240	66.03	2664	74.53
Thrip (Thysanoptera)	1747	72.32	734	52.05	2481	60.69
Little Black Ant (Monomorium minimum)	1649	47.60	414	23.29	2063	33.65
Unknown Wasp (Hymenoptera – Apocrita)	1240	82.29	482	62.74	1722	51.57
Leafhopper (Hemiptera – Cicadellidae)	384	70.85	773	69.59	1157	70.13
Wolf Spider (Arachnida – Lycosidae)	868	90.77	221	41.92	1089	62.74
Cricket (Orthoptera – Gryllidae)	796	75.28	97	18.90	893	42.92
Millipede (Diplopoda)	133	23.62	540	31.78	673	28.30
Unknown Spider (Arachnida – Araneae)	449	73.43	182	35.62	631	51.73

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on *Asclepias viridis* host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=638)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Midge (Diptera – Chironomidae)	435	25.83	160	28.22	595	27.20
Broad-nosed Weevil (Coleoptera – Entiminae)	155	37.64	315	41.37	470	39.78
Grasshopper (Orthoptera – Acrididae)	249	53.51	127	25.48	376	37.42
Jumping Spider (Arachnida – Salticidae)	175	39.48	191	37.26	366	38.21
Unkown Ant (Hymenoptera – Formicidae)	144	25.83	215	27.12	359	26.57
Darkling Beetle (Coleoptera – Tenebrionidae)	207	38.38	132	25.75	339	31.13
Stem Weevil (Coleoptera - Rhyssomatus sp.)	94	24.35	208	31.51	302	28.46
Unknown Bug (Hemiptera)	189	35.42	112	19.45	301	26.26
Tick (Arachnida – Acari)	182	17.34	111	13.97	293	15.41
Unkown Beetle (Coleoptera)	198	46.49	89	17.53	287	29.87
Harvestman (Arachnida – Opiliones)	188	25.83	89	18.63	277	21.70
Long-legged Fly (Diptera – Dolichopodidae)	93	22.88	175	26.03	268	24.69

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on *Asclepias viridis* host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=637)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Antmimic Spider (Arachnida – Salticidae)	128	25.09	50	12.60	178	17.92
Leaf Beetle (Coleoptera – Chrysomelidae)	40	11.81	133	23.84	173	18.71
Lynx Spider (Arachnida – Oxyopidae)	68	20.30	80	18.08	148	19.03
Scarab Beetle (Coleoptera – Scarabidae)	50	14.02	88	17.53	138	16.04
Pharaoh Ant (Monomorium pharaonic)	81	14.76	43	8.77	124	11.32
Grass Spider (Arachnida – Agelenopsis sp.)	78	21.03	43	10.96	121	15.25
Tachinid Fly (Diptera – Tachinidae)	39	11.81	75	15.34	114	13.84
Katydid (Orthoptera – Tettigoniidae)	33	11.07	74	10.41	107	10.69
Housefly (Diptera – Muscidae)	47	11.07	59	9.04	106	9.91
Click Beetle (Coleoptera – Elateridae)	54	14.39	51	9.59	105	11.64
Stick Insect (Phasmatodea)	22	5.90	69	10.41	91	8.49
Flea Beetle (Coleoptera – Chrysomelidae)	35	11.07	52	7.40	87	8.96
Argentine Ant (Linepithema humile)	0	0.00	69	8.49	69	4.87

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on Asclepias viridis host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=637)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Dermestid Beetle (Coleoptera – Dermestidae)	18	5.90	50	9.59	68	8.02
Ground Beetle (Coleoptera – Carabidae)	31	8.49	36	9.04	67	8.81
Centipede (Chilopoda)	42	11.07	21	5.75	63	8.02
Fleshfly (Diptera – Sarcophagidae)	34	9.23	28	4.66	62	6.60
Cranefly (Diptera – Tipulidae)	7	2.58	42	9.59	49	6.60
Adult Lepidoptera (Lepidoptera)	30	10.33	19	5.21	49	7.39
Cockroach (Blattodea)	39	11.44	9	1.92	48	5.97
Orbweaver (Arachnida – Araneidae)	13	2.58	33	7.95	46	5.66
Rove Beetle (Coleoptera – Staphylinidae)	15	5.17	22	5.21	37	5.19
Flower Weevil (Coleoptera – Baridinae)	6	2.21	31	6.03	37	4.40
Red-eyed fly (Diptera)	17	5.54	14	3.01	31	4.09
Lepidoptera Larva (Lepidoptera)	8	2.95	22	5.21	30	4.25
Snail (Gastropoda)	24	8.12	0	0.00	24	3.46

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on Asclepias viridis host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=637)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Predatory Stink Bug (Hemiptera – Asopinae)	1	0.37	20	4.38	21	2.67
Seedbug (Hemiptera – Lygaeidae)	10	2.95	11	2.47	21	2.67
Mosquito (Diptera - Culicidae)	0	0.00	21	5.21	21	2.99
Mayfly (Ephmeroptera)	0	0.00	20	5.48	20	3.14
Slug (Gastropoda)	16	5.17	0	0.00	16	2.20
Springtail (Collembola)	15	4.06	0	0.00	15	1.73
Unknown Bee (Hymenoptera - Apoidea)	0	0.00	15	4.11	15	2.36
Mirid Bug (Hemiptera – Miridae)	5	1.85	8	1.92	13	1.89
Syrphid Larva (Diptera - Syrphidae)	0	0.00	12	1.10	12	0.63
Asian Ladybeetle (Harmonia axyridis)	0	0.00	11	2.47	11	1.42
Crab Spider (Arachnida – Thomisidae)	5	1.85	4	1.10	9	1.42
Big-headed Ant (Pheidole megacephala)	0	0.00	9	0.82	9	0.47
Caddisfly (Trichoptera)	0	0.00	8	2.19	8	1.26

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on Asclepias viridis host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n= 272)		Spring 2018 (n=365)		Both Years (n=637)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Termite (Isoptera)	6	1.48	0	0.00	6	0.63
Assassin Bug (Hemiptera – Reduviidae)	3	1.11	2	0.55	5	0.79
Stinkbug (Hemiptera – Pentatomidae)	3	1.11	2	0.55	5	0.79
Lacewing (Neuroptera – Chrysopidae)	2	0.74	3	0.82	5	0.79
Hemiptera (Pseudococcidae)	0	0.00	4	1.10	4	0.63
Seven-spotted Ladybeetle (Coccinella septempunctata)	0	0.00	4	1.10	4	0.63
Goldenrod Crab Spider (Misumena vatia)	0	0.00	3	0.82	3	0.47
Nursery Web Spider (Arachnida – Pisauridae)	3	1.11	0	0.00	3	0.47
Carpenter Ant (Formicidae – Camponotus sp.)	2	0.37	1	0.27	3	0.31
Syrphid Fly (Diptera: Syrphidae)	0	0.00	3	0.82	3	0.47
Running Crab Spider (Philodromidae)	0	0.00	3	0.82	3	0.47
Earwig (Dermaptera)	2	0.74	0	0.00	2	0.31
Long-jawed Orb Weaver (Arachnida – Tetragnathidae)	2	0.74	0	0.00	2	0.31

Appendix 3 Continued. Arthropods captured in sticky traps adjacent to focal monarch eggs on Asclepias viridis host plants. Sample sizes in parentheses refer to the number of eggs associated with each type of arthropod.

	Spring 2017 (n=272)		Spring 2018 (n=365)		Both Years (n=637)	
	Number Captured	Percent Frequency	Number Captured	Percent Frequency	Number Captured	Percent Frequency
Leather Bug (Hemiptera – Coreoidea)	2	0.74	0	0.00	2	0.31
Scorpionfly (Mecoptera)	2	0.74	0	0.00	2	0.31
Paper Wasp (Polistes sp.)	0	0.00	2	0.27	2	0.16
Diptera (Liriomyza sp.)	0	0.00	2	0.55	2	0.31
Long-necked Seed Bug (Myodocha serripes)	0	0.00	1	0.27	1	0.16
Borderbug (Hemiptera – Lygaeidae)	1	0.37	0	0.00	1	0.16
Large Milkweed Bug (Oncopeltus fasciatus)	1	0.37	0	0.00	1	0.16
Plecoptera	0	0.00	1	0.27	1	0.16
Scorpion (Arachnida - Scorpiones)	0	0.00	1	0.27	1	0.16

Appendix 4. Definitions, counts, and percent frequency of arthropod groups captured in traps around host plants and used in statistical analyses. Data based on occurrences in traps associated with 638 monarch eggs or larvae on 409 host plants.

Arthropod Group	Included Taxa	Raw Count	Percent Frequency
Formicidae, *Solenopsis invicta*	RIFA, Formicidae, *Solenopsis invicta*	13007	78.8
Diptera < 5 mm	Midges (Chironomidae), Mosquitoes (Culicidae), unknown flies (Diptera) < 5 mm body length	7009	92.9
Custacea: Isopoda	Isopods	5554	62.0
Hemiptera, Aphididae	Aphids (Aphididae)	4904	91.1
Arachnida, Acari	Mites and Ticks (Arachnida, Acari)	2957	78.8
Thrips (Thysanoptera)	Thrips (Thysanoptera)	2481	60.6
Formicidae, *Monomorium minimum*	Little Black Ants, Formicidae, *Monomorium minimum*	2063	33.6
Hymenoptera, Apocrita < 5 mm	Wasp (Hymenoptera – Apocrita), < 5 mm body length	1646	69.2
Lycosidae, Agelenidae, Pisuridae	Wolf (Lycosidae), Grass (Agelenidae), Nursery Web Spiders (Pisuridae)	1213	66.2
Auchenorrhyncha	Leafhoppers (Hemiptera,Auchenorrhyncha)	1157	70.0
Other Predators	Rove Beetles (Staphylinidae), Ground Beetles (Carabidae), Assassin Bugs (Reduviidae), Predatory Stink Bugs (Pentatomidae, Asopinae), other Ants (Formicidae), Vespid Wasps (Vespidae), Centipedes (Chilipoda), Long-legged Flies (Dolichopodidae), Scorpionflies (Mecoptera), Lacewings (Neuroptera), Hoverflies (Adults and larvae, Syrphidae), Lady Beetles (Coccinellidae), and Scorpions (Scorpiones).	1141	67.8

Appendix 4 continued. Definitions, counts, and percent frequency of arthropod groups captured in traps around host plants and used in statistical analyses. Data based on occurrences in traps associated with 638 monarch eggs or larvae on 409 host plants.

Arthropod Group	Included Taxa	Raw Count	Percent Frequency
Orthoptera – Gryllidae	Crickets (Orthoptera – Gryllidae)	893	42.9
All Other Arthropods	Stick Insects (Phasmatodea), Click Beetles (Elateridae), Leaf-footed Bugs (Coreidae), Seed Bugs (Lygaeidae), Plant Bugs (Miridae), Shield Bugs (Pentatomoidea, non-predatory), unknown bugs (Hemiptera), Springtails (Collembola), Butterflies, Skippers, Moths (Lepidoptera), Slugs and Snails (Mollusca), Crane Flies (Tipulidae), Termites (Isoptera), Earwigs (Dermaptera), Roaches (Blattodea), Caddisflies (Trichoptera), Stonefly (Plecoptera), Mayflies (Ephemeroptera), and Unknown Bees (Anthophila)	824	60.4
Araneae, Others	Long-jawed Orb Weavers (Tetragnathidae), Jumping Spiders (Salticidae), Lynx Spiders (Oxyopidae), Crab Spiders (Thomisidae), Running Crab Spiders (Philodromidae), and unidentified spiders > 5 mm body length	820	61.9
Coleoptera, Curculionidae	Coleoptera: All Weevils (Curculionidae)	809	57.3
Millipedes (Diplopoda)	Millipedes (Diplopoda)	673	28.3
Calyptrate Flies	Diptera: Flesh Flies (Sarcophagidae), Tachinid Flies (Tachinidae), House Flies (Muscidae), and unknown Calyptratae	576	47.4
Araneae < 5 mm	Unidentified Spiders < 5.0 mm body length	566	47.7
Scavenging Beetles	Coleoptera: Tenebrionidae, Scarabaeidae, Dermestidae	545	46.0

Appendix 4 continued. Definitions, counts, and percent frequency of arthropod groups captured in traps around host plants and used in statistical analyses. Data based on occurrences in traps associated with 638 monarch eggs or larvae on 409 host plants.

Arthropod Group	Included Taxa	Raw Count	Percent Frequency
Orthoptera, Caelifera and Tettigoniidae	Grasshoppers (Caelifera) and Katydids (Tettigoniidae)	483	42.1
Coleoptera, < 10 mm	Coleoptera, unidentified < 10 mm body length	287	29.8
Harvestmen (Opiliones)	Harvestmen (Opiliones)	277	21.7
Chrysomelidae	Coleoptera: Flea beetles (Chysomelidae, Alticini), other leaf beetles (Chysomelidae)	260	26.4